CALIFORNIA FISH and GAME

"CONSERVATION THROUGH EDUCATION"

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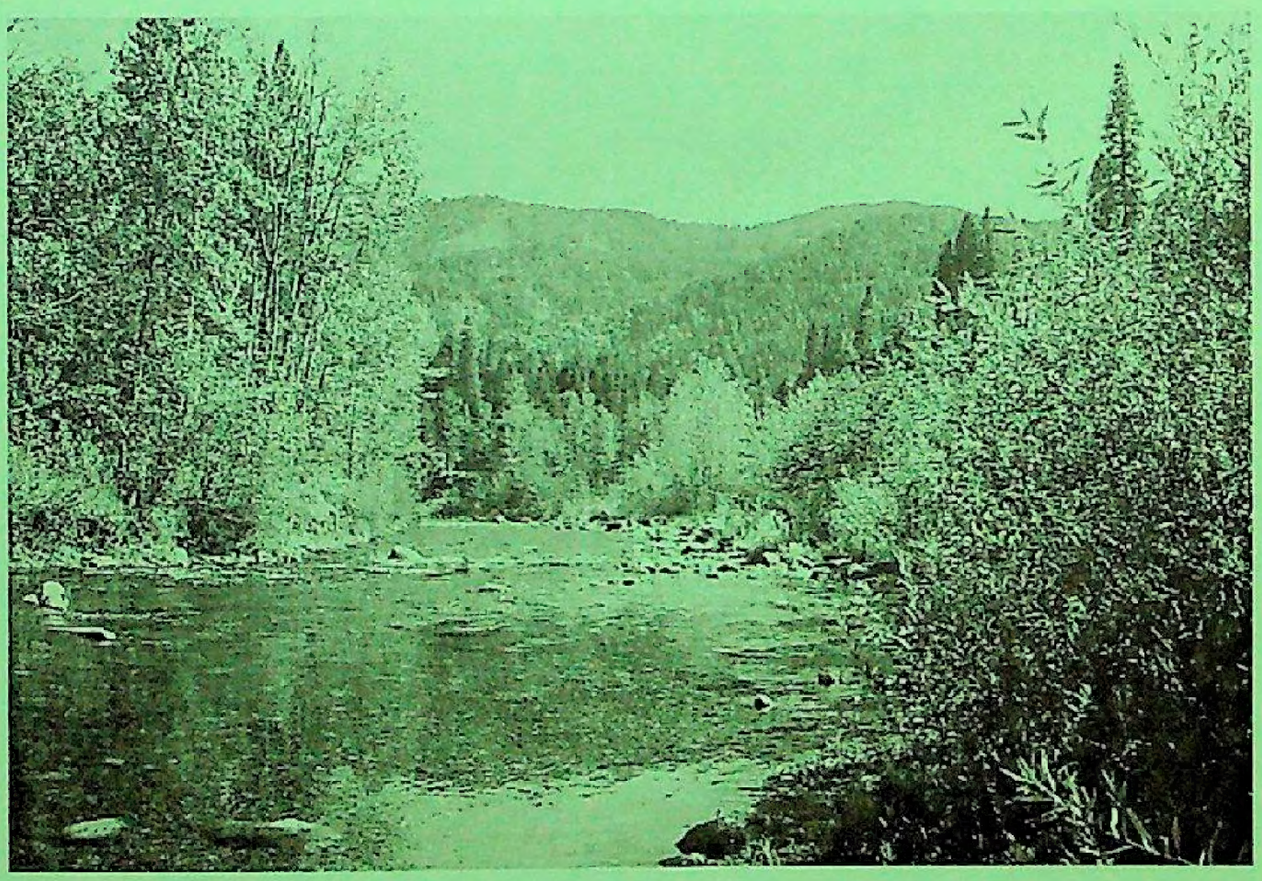

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Sacramento River at Soda Creek
California Department of Fish and Game Cantara Program
Photographer: J. Miller; 20 September 1992

EDITORS' NOTE

This issue of *California Fish and Game* is dedicated to papers describing various aspects of the unfortunate Cantara toxic spill of 1991 in the upper Sacramento River, California's largest river. This is an ecologically and recreationally important area which supports a valuable sport fishery for native rainbow trout. Barrett Garrison served as special editor for this issue, soliciting and editing papers from among those presented at the February 1997 Cantara Spill Symposium and encouraging authors over the long road to publication. We extend our special thanks to him.

California Fish and Game 86(1):1-3 2000

SYMPOSIUM OVERVIEW: DAMAGE TO AND RECOVERY OF AQUATIC AND RIPARIAN ECOSYSTEMS IN THE UPPER SACRAMENTO RIVER FROM THE CANTARA CHEMICAL SPILL

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On 14 July 1991, a Southern Pacific Transportation Company railroad train derailed along the Sacramento River in Siskiyou County, California at a location known as the "Cantara Loop." A chemical tank car discharged 72,000 liters of the soil fumigant metam sodium into the Sacramento River. The metam sodium and its breakdown products, including methyl isothiocyanate, killed virtually all aquatic life in the river from the Cantara Loop downstream for approximately 58 km. The herbicide was eventually diluted, dissipated, and broken down in Shasta Lake. Riparian plant communities along the river also suffered extensive damage, as did many wildlife species dependent on the river and its riparian vegetation. Disastrous as the Cantara spill was for river-dependent biota, the ecosystem is recovering.

A federally mandated Natural Resource Damage Assessment (NRDA) identified and quantified impacts to natural resources so that the Southern Pacific Transportation Company and other responsible parties would compensate for the losses. Post-spill studies of the Sacramento River ecosystem provided important information regarding damage to and recovery of riverine ecosystems following a chemical spill. Furthermore, the spill provided insight into practical challenges associated with large-scale investigations of ecological disasters.

Several actions were taken to assist recovery of aquatic and riparian life along the river (CDFG[1] 1999). One action aimed at re-establishing trout, *Oncorhynchus* spp., populations was a moratorium on angling for 3 years. Physical and operational changes to railroad facilities have improved the safety of trains passing through the upper Sacramento River canyon and Cantara Loop. A set of metal rails was placed inside the original tracks in the Cantara Loop to prevent derailed cars from leaving the rail bed and falling into the river. Additionally, the number of engines required has been increased, and the speed at which trains are allowed to negotiate this turn has been lowered to help prevent another derailment. The Cantara Trustee Council was created shortly after the spill to provide resources, assist recovery and research, and administer settlement monies from the suit against the responsible parties. To date, US$38 million has been obligated from the settlement.

[1] CDFG. 1999. Draft fishery management plan for the Upper Sacramento River (Box Canyon Dam to Lake Shasta) 2000 to 2005, Shasta/Siskiyou counties, California. California Department of Fish and Game, Northern California-North Coast Region, Redding, California, USA.

The 6 papers in this issue of *California Fish and Game* resulted from 16 presentations at a symposium held on 12 February 1997 in Sacramento, California that addressed the Cantara spill. Sponsored by the California Department of Fish and Game, Cantara Trustee Council, and Western Section of The Wildlife Society, the symposium was the 1st comprehensive presentation of the monitoring that documented initial damage to and recovery of terrestrial and aquatic components from the spill. Over 150 people attended the symposium, including resource managers, scientists, anglers, river conservationists, and ecosystem restorationists.

The papers cover important issues directly associated with the spill. Stopher (2000) analyzes the challenging NRDA process and discusses the lessons learned. Sheehy et al. (2000) describe a decision-analysis method that used natural resource and societal attributes to rank and prioritize restoration efforts for funding. Hankin and McCanne (2000) estimate the number of fish killed by the spill and the proportions of wild and hatchery rainbow trout, *Oncorhynchus mykiss*, killed. Genetic identities of populations of rainbow trout that recolonized the Sacramento River after the fish kill are examined by Nielsen et al. (2000). Luke and Sterner (2000a, b) find that amphibians and reptiles were variously affected by the spill.

Information presented in these papers shows that damage to fish and wildlife from the spill was extensive, but that some recovery has already occurred and more is in progress. The cost of this recovery in dollars and human effort has been substantial (Sheehy 2000, Stopher 2000). Providing scientifically valid and legally defensible quantification of spill impacts proved challenging to scientists conducting the NRDA. Baseline ecological data were essentially nonexistent and mobilizing qualified scientists to begin immediate impact assessments was difficult because natural resource agencies were already committed to other tasks and a program was not in place. Ecological disasters like the Cantara Spill teach us that riverine ecosystems are both fragile and resilient, and that baseline information on natural resources has untold value should these ecological disasters occur. My hope is that the information presented in this issue can 1) help prevent future chemical spills so that fish and wildlife resources are not impacted, 2) improve natural resource assessments should spills unfortunately occur again, and 3) provide justification for conducting baseline natural resource assessments so our ability to document impacts and calculate compensation will improve.

ACKNOWLEDGMENTS

I thank M. Stopher and S. Turek for their helpful reviews of this paper. D. Carrier, B. Hull, C. Martz, J. Miller, J. Nelson, M. Stopher, and S. Turek deserve appreciation for their help organizing and conducting the symposium. I also thank the individuals who conducted excellent reviews of the papers from the symposium.

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California Fish and Game 86(1):4-20 2000

ESTIMATING THE NUMBER OF FISH AND CRAYFISH KILLED AND THE PROPORTIONS OF WILD AND HATCHERY RAINBOW TROUT IN THE CANTARA SPILL

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Following the 1991 Cantara toxic spill on the upper Sacramento River, California Department of Fish and Game employees carried out 2 kinds of surveys to allow estimation of the total fish kill: 1) extensive shoreline visual counts of dead fish, and 2) dipnet collections of carcasses. An additional experiment, consisting of shoreline visual counts, followed by dipnet collections, and finally snorkel collections of dead fish, allowed estimation of species-specific fractions of carcasses that were observed in shoreline counts. We used reach-stratified ratio estimation methods to produce 2 alternative estimates of the total number of fish killed and of the distribution of these fish kills across species categories. These 2 methods produced estimated total fish kills of 1.125 million or 1.137 million fish, respectively, mostly riffle sculpins, *Cottus gulosus*, (655,000 or 749,000 fish) and trout (mostly rainbow trout, *Oncorhynchus mykiss)* (309,000 or 236,000 fish). These estimates are conservative. We used linear discriminant analysis and distribution mixture separation methods to estimate the fractions of wild fish among trout kills, relying on differences in means and coefficients of variation of circuli spacing on scales of wild and hatchery fish. Discriminant analysis classified 85% of trout kills as wild fish, but this estimated proportion has substantial negative bias. When distribution separation methods were applied to discriminant scores for dead trout, estimated wild fish proportions ranged from 90–96%. These high estimated wild fish proportions are similar to a previous estimate, based on body morphology and coloration, that 90% of trout were wild during 1980–1981, and to a contemporary estimate, based on genetic methods, that 83% of rainbow trout killed in the 1991 Cantara Spill were wild.

INTRODUCTION

On 14 July 1991, a Southern Pacific train derailment at the Cantara Loop on California's upper Sacramento River resulted in release of metam sodium, a soil fumigant. This toxic spill killed all aquatic life from the Cantara Loop downstream to Shasta Lake, a distance of approximately 58 km (Fig. 1). Of particular concern were kills of rainbow trout, *Oncorhynchus mykiss*, which had supported a substantial and highly regarded "blue ribbon" recreational fishery (Sunderland and Lackey

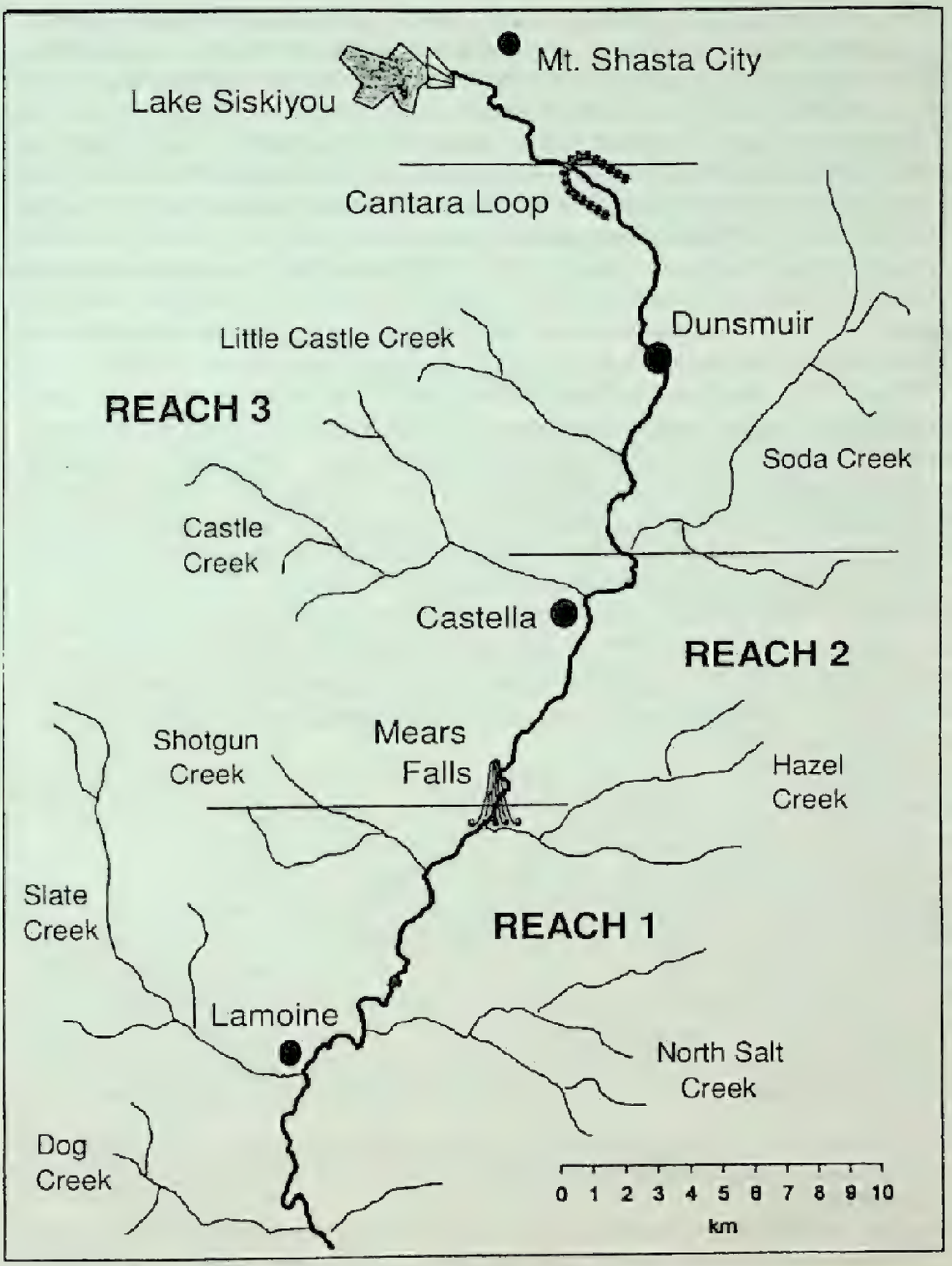

Figure 1. Map of the upper Sacramento River showing the 3 reaches used in post-stratified ratio estimation of the total fish kill following the Cantara Spill.

1998). According to Snider and Linden[1] (1981), back-calculated total lengths of these rainbow trout at ages 4 and 5 averaged about 38.9 cm and 45.5 cm, respectively, lengths comparable to rainbow trout from other California blue ribbon trout streams (such as the McCloud River, Pit River, and Hat Creek).

The devastating 1991 spill had been preceded by a lesser toxic spill resulting from a Southern Pacific train derailment at the same Cantara Loop location on 10 April 1976. This earlier spill resulted in the release of unspecified toxic materials that killed at least 6,500 wild rainbow trout and an unknown number of nongame fish and invertebrates (Rode and Zuspan[2] 1993). With funds from the settlement for the 1976 spill, California Department of Fish and Game (CDFG) biologists carried out studies (1980–1981) designed, among other things, to determine the contribution of wild and hatchery rainbow trout to the overall fish population and to the fishery.

Prior to 1982, the upper Sacramento River had been managed as a "put-and-take" catchable trout stream, with approximately 80,000–90,000 catchable trout stocked annually, a 10-fish limit, and no special gear restrictions. The 1980–1981 CDFG studies suggested, however, that although catchable hatchery trout made substantial short-term contributions to recreational fisheries (about 90% of catches were made within 4 weeks of planting), they made little contribution to the overall population or to the trophy (> 35.6 cm total length) rainbow trout fishery. Wild rainbow trout (distinguished from hatchery fish on the basis of coloration, body form, and fin erosion or deformity) made up about 90% of all trout sampled in the 1980–1981 studies, and virtually no fish >30.5 cm were of hatchery origin, thus implying that non-harvested catchable trout experienced poor winter survival (Rode and Zuspan[2] 1993, T.R. Payne and Associates[3] 1995).

Beginning with the 1983 trout season, new angling regulations for trout were adopted with the intention of fostering a catch-and-release attitude among anglers and promoting and protecting the trophy wild trout fishery. Stocking of catchable trout was continued in the upper 40.7 km of the upper Sacramento River, where the daily limit of 10 trout remained unchanged, but stocking of catchable trout was eliminated in the lower 22 km and a 2-trout bag limit was imposed in this "wild trout" area. Based on 1986 creel survey results (T.R. Payne and Associates 1995), these regulation changes resulted in fewer rainbow trout being caught, but a substantial increase in the percentage of large (> 35.6 cm) wild trout in the creel. Trout fishery regulations at the time of the 1991 spill were similar, except that the daily limit had been reduced to 5 fish in the area stocked with catchable trout.

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[1] Snider, W.M. and A. Linden. 1981. Trout growth in California streams. Inland Fisheries Administrative Report No. 81-1. California Department of Fish and Game, Sacramento, California, USA.

[2] Rode, M. and M. Zuspan. 1993. Upper Sacramento River fishery investigations, part I: Fish population, harvest rate and migration studies. Unpublished draft Inland Fisheries Administrative Report, California Department of Fish and Game, Sacramento, California, USA.

[3] T.R. Payne and Associates. 1995. Upper Sacramento River creel censuses, 1978–1986. Prepared for California Department of Fish and Game, Cantara Program, Redding, California.

In the days immediately following the 1991 Cantara Spill, CDFG biologists collected samples of dead fish and crayfish from the Cantara Loop downstream to Shasta Lake. The intention of these collections was to allow description of the magnitude and species composition of the kill of fish and crayfish (hereafter, "fish kill"). Based on these collected data, we use post-stratified ratio estimation methods to estimate the total number of rainbow trout, nongame fish and crayfish killed in the 1991 spill. We also used discriminant analysis and distribution mixture separation methods to separate rainbow trout kills into hatchery and wild fish on the basis of scale patterns reflecting 1st year growth.

METHODS

Post-Spill Data Collections

Due to concerns over worker safety in the spill area (exposure to toxic fumes), substantial post-spill survey efforts were not initiated until 18 July 1991, 4 days after the spill. From 18 to 21 July 1991, workers under the supervision of CDFG biologists carried out 2 distinct types of surveys: 1) shoreline visual counts of dead fish, and 2) dipnet collections of dead fish. In addition, CDFG workers carried out the so-called "Conant Experiment," designed to establish the relationship between shoreline counts and the true number of dead fish.

Shoreline visual counts of dead fish were made to an imaginary mid-channel line along 600–1200-m reaches of the east and west banks of the upper Sacramento River at approximate river kilometers (rkm) (above Shasta Lake) 1.6, 9.7, 11.3, 14.5, 25.7, 35.4, 37.0, 43.4, 48.3, and 53.1 on 18–20 July 1991. Survey reaches were selected purposively, in part on the basis of accessibility, but also to coincide with reaches that CDFG workers had surveyed by electrofishing in previous years. Visual counts were made from a total of approximately 16,500 m of shoreline (east and west banks combined), as compared to the total spill-affected shoreline of approximately 119,000 m (east and west banks combined), and accounted for about 14% of affected stream length. Within surveyed reaches, workers were instructed to record the following data: 1) habitat type (pool, run, or riffle); 2) "percent seen"; 3) unit length (measured by hip chain); and 4) numbers of dead fish by 1 of 7 categories that could be accurately separated by shoreline observation: trout (almost exclusively rainbow trout, but also probably a few brown trout, *Salmo trutta*; and brook trout, *Salvelinus fontinalis*); sucker (Sacramento sucker, *Catostomas occidentalis*); pikeminnow-hardhead (Sacramento pikeminnow, *Ptychocheilus grandis*, and hardhead, *Mylopharodon conocephalus*); sculpin (riffle sculpin, *Cottus gulosus*); crayfish (signal crayfish, *Pasifastacus leniusculus*); bass (smallmouth bass, *Micropterus dolomieu*, and spotted bass, *M. punctulatus*); and unidentifiable fish.

"Percent seen" was a subjective valuation of the percentage of the total area of a given habitat unit in which workers believed that they could effectively count dead fish. Surface glare, turbulence, depth, bank access, and other factors all contributed to reduce percent seen from its maximum value of 100%. When it was impossible to obtain a visual count of dead fish along the shoreline (e.g., inaccessible bank),

workers were instructed to record the length of such a section and to list the percent seen as 0%.

Dipnet collections of all dead fish that could be located and reached while wading (in chest waders) were made on 19–21 July 1991 using long-handled (approximately 1.5 m) dipnets along approximately 180–250-m reaches of shoreline at approximate rkm 1.6, 11.3, 25.7, 35.4, 37.0, 41.8, and 48.3. Locations for these collections coincided roughly with those of shoreline counts that had been made on the previous day. Generally, no data sheets were kept for these collections and counts of fish by species categories were not recorded. Instead, collected dead fish (all species) were placed in labeled plastic bags and frozen.

In the 22 July 1991 "Conant Experiment", an approximately 290-m reach of stream (at Conant Road, rkm 34.0) containing a typical sequence of 2 pools, 2 riffles, and 2 runs was fenced off, using chicken wire, to prevent fish carcasses from entering or leaving the experimental area. On the morning of 22 July 1991, field workers made visual counts of fish from the east and west shorelines, following described protocols for shoreline counts. Following the shoreline counts, teams made dipnet collections of dead fish while wading, collecting all of the carcasses that could be collected in this fashion. Carcasses were counted, measured, identified by species category and habitat type, and placed in labeled plastic bags. Following these dipnet collections, a team of divers, wearing wet suits, masks, and snorkels, collected remaining dead fish. These fish were counted by identified habitat unit, but were not assigned to fish categories. The calculated ratio of 1) the total number of fish collected by dipnet plus diving compared to 2) the number of fish recorded in shoreline counts was intended to allow adjustment (expansion) of recorded shoreline counts to actual fish kills.

Estimation of Fish Kills

We used visual counts with and without results from the Conant Experiment to produce 2 alternative sets of estimates for the numbers of fish killed, identified by species. Our estimates were based on treating the survey data as if they had been collected using a stratified random sampling design with stratification by stream reach (see below). In a very few instances, data recorded by certain field workers at specific locations appeared unreliable or unusable due to vaguely specified counts or values (e.g., when trout or sculpin numbers were reported as "numerous" or when relative numbers of trout and sculpin were reported as 40% and 60% rather than as numerical values). These data records were excluded from calculations.

Both strategies used to estimate numbers of fish killed were post-stratified ratio estimation approaches (see Cochran 1977), but they differed in their use of percent seen values recorded by field workers. For the 1st strategy, recorded visual counts of fish in particular habitat units were first adjusted upwards by corresponding percent seen values for those units; we refer to such adjusted values as "expanded counts." The resulting estimated numbers of fish killed did not rely in any manner on results from the Conant Experiment. For the 2nd strategy, the recorded visual counts of

fish in particular habitat units were not adjusted by percent seen values, but were instead adjusted upwards using expansion factors based on the Conant Experiment.

For each ratio estimation approach, calculations began with summation of the observed (or expanded) counts within a particular stratum, and then division of that sum by the corresponding sum of all sampled habitat unit shoreline lengths in that stratum, thus producing, for each species category (including unidentified fish), a calculated ratio of dead fish (observed or expanded numbers) compared to the total length of counted stream shoreline in a particular stratum. Throughout the following text, we refer to such calculated ratios as "densities" to simplify interpretation of our methods. Calculated densities were then multiplied by 2 times total reach length to generate estimates of the total (observed or expanded) number of dead fish in that reach. The adjustment by a factor of 2 accounted for the fact that shoreline counts were made for half-stream widths rather than full-stream widths.

The estimation methods used stream reach (Fig. 1) as a stratification factor. Choice of stream reach as the stratification factor was based on assessment of variation in densities (based on expanded counts) of species categories by reach as compared to variation in density by survey date (18, 19, or 20 July 1991) or by habitat type (riffle, run, or pool) if used as alternative stratification factors. For most species categories, variation in densities across reaches was greater than variation across survey dates or habitat types, thus supporting selection of reach as the stratification factor (see Results). The boundaries of Reach 1 were Shasta Lake (rkm 0) and Mears Falls (rkm 27.8), a possible barrier to upstream migration of suckers, pikeminnow, hardhead, and bass; the upper boundary of Reach 2 was Soda Creek (rkm 42.0); and the upper boundary of Reach 3 was the Cantara Loop itself (rkm 59.5).

In mathematical notation, the stratified estimators can be described as follows: Let y_{ij} denote the observed count of fish (by species category) in habitat unit i surveyed in Reach j (j = 1,2,3); let z_{ij} denote the recorded percent seen value for habitat unit i in Reach j; and let x_{ij} denote the corresponding recorded shoreline length of habitat unit i in Reach j. Let n_j denote the total number of surveyed habitat units in Reach j; and let X_j denote the total length of Reach j. The estimated ratio of observed (unexpanded) counts to surveyed shoreline of stream in Reach j is

$$\hat{R}_{Uj} = \sum_{i}^{n_j} y_{ij} \Big/ \sum_{i}^{n_j} x_{ij}$$

and the estimated ratio of expanded counts to surveyed length of stream shoreline in Reach j is

$$\hat{R}_{Ej} = \frac{\sum_{i}^{n_j} y_{ij} / z_{ij}}{\sum_{i}^{n_j} x_{ij}}$$

(Note that similar ratios (densities) were calculated for alternative stratification variables-habitat types or survey dates, as discussed above.) Finally, totals for Reach j were estimated as

$$\hat{Y}_{Uj} = 2X_j\hat{R}_{Uj} \text{ and } \hat{Y}_{Ej} = 2X_j\hat{R}_{Ej}$$

where subscripts U and E refer to unexpanded and expanded counts.

Data from the Conant Experiment were used to make an alternative adjustment to the observed shoreline counts that did not rely on the subjective percent seen values. Unidentified fish in shoreline visual counts were allocated to species categories, excluding crayfish, using observed percentages among fish assigned to identifiable species categories. Because fish collected by divers were counted but not identified by species category (except for crayfish), species category percentages in dipnet collections were used to allocate snorkel counts to identified species categories (excluding crayfish). Species-specific expansion factors based on Conant Experiment data were calculated essentially by taking the sum of the dipnet and diver collections of dead fish (by species category), assumed equal to the true number of dead fish present on 22 July 1991, and dividing these totals by allocated numbers of fish assigned to species categories in corresponding shoreline counts. Because no suckers or bass were recorded in shoreline counts from the Conant Experiment, we could not calculate expansion factors for these species. Instead, we assumed that the expansion factor calculated for pikeminnow-hardhead was also appropriate for suckers and bass. These species-specific expansion factors, z_k, were used as multipliers to adjust estimated total unexpanded shoreline counts, thus generating alternative estimates of total fish kills, $\hat{Y}'_{Ej}$, that should have been comparable to the $\hat{Y}_{Ej}$: $\hat{Y}'_{Ej} = z_k\hat{Y}_{Uj}$.

Estimation of the Proportion of Wild Rainbow Trout

We used linear discriminant analysis (Tatsuoka 1970, Stevens 1986) to classify dead trout into hatchery or wild categories based on scale patterns of trout collected by dipnet following the Cantara Spill. We defined wild trout as trout produced from natural spawning of rainbow trout in the upper Sacramento River or its tributaries, whereas hatchery fish were artificially spawned and reared in a hatchery prior to release. Typically, a discriminant function is constructed from baseline data drawn from each of 2 categories, where category membership is known with certainty. This function is then used to classify individuals in an independent sample drawn from a population containing an unknown mixture of the 2 categories. Our baseline sample of known hatchery fish consisted of scales taken from 47 Shasta strain rainbow trout reared at Shasta Hatchery during 1991. Scale patterns from these fish should be essentially the same as those for hatchery fish that had been stocked in 1991. For our wild sample, we took scales from 170 dipnet collected rainbow trout with fork lengths $\leq$178 mm that were collected following the Cantara Spill. Catchable rainbow trout stocked in the upper Sacramento River averaged about 255 mm and essentially none would have been $\leq$178 mm at time of stocking (R. Corn, CDFG, personal communication).

Because hatchery fish rear in a controlled environment that has greater stability in temperature and food supply than the environment of wild fish, we reasoned that differences between scale growth patterns of wild and hatchery fish should allow separation of the 2 types using discriminant analysis (e.g., Unwin and Lucas 1993). *A priori*, we anticipated that average distance between adjacent circuli would be larger and variation in circuli spacing would be less for hatchery fish than for wild fish. As discriminant variables, we used 1) mean circuli spacing for the first 15 circuli and 2) coefficient of variation (CV) of circuli spacing for the first 15 circuli. These early circuli should reflect growth conditions for juvenile fish. Scale measurements were made in standardized sampling units on a computer-based imaging system from the focus to each circulus on a line 30° from a vertical axis drawn through the scale focus to the anterior edge of the scale.

We assessed accuracy of classification by calculating apparent misclassification probabilities (Stevens 1986) among our baseline samples. We then used our constructed discriminant functions to classify trout as wild or hatchery in a large sample of 586 fish collected following the Cantara Spill. These fish had been collected during the Conant Experiment (rkm 34.0) and also from dipnet collections made at rkm 1.6, 11.3, 25.7, 35.4, 37.0, 41.8, and 48.3. Sample sizes at individual locations were variable (11–119 fish) and generally too small to warrant site-specific estimation of hatchery fish proportions. The large combined sample included those fish ≤178 mm that were used to construct the discriminant function, but we did not assume that all such fish were wild when we applied discriminant analysis procedures; some of these small, presumptively wild, fish could have been classified as hatchery fish.

When the true proportion of 1 category is much greater than the proportion belonging to the alternative category, discriminant analysis can produce a much greater number of misclassifications for the larger category than for the smaller category (see Results), thus creating substantial bias in estimated proportions. Therefore, we applied McDonald and Pitcher's (1979) methods for separation of distribution mixtures to the raw discriminant scores associated with each fish in the unknown sample, including and excluding those fish ≤178 mm. We estimated proportions of wild and hatchery fish given each of 3 alternative assumptions regarding true discriminant scores for the 2 component groups: 1) true means and variances are known; 2) variances are equal, but neither means nor variances are known; or 3) variances are unequal and neither means nor variances are known. We also calculated chi-square goodness-of-fit values to judge whether or not results from the distribution mixture separation method produced an adequate description of the distribution of discriminant scores for the 2 component groups (hatchery and wild).

RESULTS

Estimated Fish Kills

Variation in densities of individual fish species categories (based on expanded counts) was greater across reach strata than across alternative habitat type or survey

date strata, thus supporting our use of stream reach as a stratification variable (Table 1). Suckers, pikeminnow-hardheads, and bass were largely restricted to Reach 1 (closest to Shasta Lake), with greatly reduced densities in Reach 2 and absence in Reach 3. Trout and sculpins had relatively uniform densities across reaches, whereas densities of unidentified fish and crayfish were much lower in Reach 3 than in Reaches 1 and 2. In contrast, with the exception of sculpins, for which densities in pools were greater than in other habitat unit types, densities of species categories showed little variation with habitat type. Variation in densities across sampling date was substantial, but appeared to reflect variation in reach sampled on different dates rather than an effect of sample date itself on densities. Densities of nearly all species categories except trout were greater on 19 July than on 18 or 20 July, and densities of pikeminnow-hardhead, suckers, and bass were lowest on 18 July. Field workers were largely restricted to the upper river (Reaches 2 and 3) on 18 July, thus explaining the extremely low densities of pikeminnow-hardheads, suckers, and bass on that day. Similarly, the high densities for these same species and generally high densities for other species on 19 July probably reflected the fact that all surveys on 19 July were in Reach 1, where densities of nearly all species categories were high.

When visual shoreline counts were expanded by percent-seen values, the reach-stratified ratio estimate for total kill of all species of fish and crayfish was 1,128,520.

Squawfish-hardhead, sucker, and bass kill estimates were dominated by Reach 1, and crayfish estimates were almost entirely from Reaches 1 and 2; substantial kills of trout and sculpin occurred in all 3 reaches (Table 2). Unidentified fish accounted for about 42% of the estimated total fish kill, and almost 70% of these unidentified fish were from Reach 1. When unidentified fish were allocated to species categories in each reach, estimated total kills were 655,160 sculpins, 312,508 trout, 74,161

Table 1. Estimated densities (expanded visual shoreline counts per meter) by species categories for fish killed in the 1991 Cantara Spill, stratified by reach (see Fig. 1), habitat type, and survey date.

	Unidentified fish	Trout	Sucker	Pikeminnow-hardhead	Sculpin	Crayfish	Bass
			Stratified by reach				
Reach 1	0.5994	0.1070	0.0296	0.0571	0.3111	0.0509	0.0022
Reach 2	0.3778	0.2211	0.0031	0.0117	0.2212	0.0612	0.0000
Reach 3	0.0881	0.1416	0.0000	0.0000	0.3708	0.0027	0.0000
			Stratified by habitat type				
Pools	0.5157	0.1315	0.0116	0.0500	0.4467	0.0530	0.0007
Riffles	0.4184	0.1713	0.0137	0.0214	0.0259	0.0418	0.0006
Runs	0.3911	0.1426	0.0164	0.0298	0.2639	0.0544	0.0016
			Stratified by survey date				
July 18	0.4249	0.2651	0.0035	0.0124	0.3200	0.0567	0.0000
July 19	0.6425	0.1521	0.0349	0.0930	0.4160	0.0668	0.0007
July 20	0.3068	0.0728	0.0119	0.0086	0.1877	0.0274	0.0020

Table 2. Estimated total number of fish killed in the upper Sacramento River by the 1991 Cantara Spill based on visual shoreline counts expanded by recorded percent-seen values. The Unallocated section lists unidentified fish as a separate species category, whereas the Allocated section allocates the unidentified fish according to reach-specific percentages of total identified species (excluding crayfish) by reach.

Stratum	Unidentified fish	Trout	Sucker	Pikeminnow-hardhead	Sculpin	Crayfish	Bass	Total
Unallocated								
Reach 1	325,032	58,008	16,052	30,980	168,748	27,590	1,174	627,584
Reach 2	115,586	67,648	970	3,590	67,676	18,724	0	274,194
Reach 3	33,102	53,234	0	0	139,386	1,020	0	226,742
Total	473,720	178,890	17,022	34,570	375,810	47,334	1,174	1,128,520
Allocated								
Reach 1		126,580	35,027	67,601	368,224	27,590	2,562	627,584
Reach 2		123,546	1,768	6,560	123,596	18,724	0	274,194
Reach 3		62,382	0	0	163,340	1,020	0	226,742
Total		312,508	36,795	74,161	655,160	47,334	2,562	1,128,520

pikeminnow-hardhead (combined category), 47,334 crayfish, 36,795 suckers, and 2,562 bass (Table 2).

Expansion factors calculated from data generated in the Conant Experiment ranged from 6.47 (sculpin) to 3.86 (trout) (Table 3). Therefore, in the Conant experimental reach, shoreline counts represented from 15% to 26% of the total number of dead fish that could be collected.

When visual shoreline counts were expanded upward using these species-specific expansion factors, the estimated total kill of fish and crayfish was 1,136,938 (Table 4). Estimated total kills by species category in this case were 749,458 sculpins, 236,630 trout, 73,800 squawfish-hardhead, 45,358 crayfish, 29,060 suckers, and 2,632 bass. Patterns of relative fish kills by species in different reaches were consistent with those patterns generated from use of visual counts expanded by percent-seen values: bass, sucker and pikeminnow-hardhead kills originated almost exclusively from Reach 1; estimated crayfish kills were dominated by kills in Reaches 1 and 2; and estimated kills of trout and sculpin were substantial in all 3 reaches (Table 4).

Estimated Proportion of Wild Rainbow Trout

When CV of circuli spacing and mean circuli spacing for the first 15 circuli were plotted as (x,y) coordinates of points, it was apparent that CV of circuli spacing was usually smaller and mean spacing between circuli was usually larger for hatchery fish than for wild fish (Fig. 2). When the constructed linear discriminant function was used to classify the baseline wild fish sample, 12 of 170 presumptively wild fish were misclassified as hatchery fish, giving an apparent misclassification rate of approximately 7%. When the constructed discriminant function was applied to the

Table 3. Calculations of species category-specific "expansion factors" from results of Conant Experiment. Total shoreline in the Conant reach (east and west sides combined) was approximately 579 m, whereas shoreline counts, made to an imaginary midline of the river, could only be made for approximately 496 m of shoreline.

	Unidentified fish	Trout	Sucker	Pikeminnow-hardhead	Sculpin	Crayfish
Total shoreline count	176	103	0	9	123	27
Count per meter	0.355	0.208	0	0.018	0.248	0.054
Adjusted total count[a]	205	120	0	11	144	32
Allocated total count[b]		209	0	19	251	32
Dipnet + allocated snorkel collections[c]		806	5	89	1,624	189
Expansion Factor[d]		3.856	—	4.684	6.470	5.906

[a] Count per m x 579 m.

[b] Unidentified fish were allocated to species categories following species category distribution for identified fish.

[c] Sum of numbers of fish collected by dipnet (identified by species category) and numbers of fish collected by snorkeling (not identified by species, but allocated following species category distribution of dipnet collected fish).

[d] Dipnet + allocated snorkel collections divided by allocated total count.

Table 4. Calculated total number of fish killed in the 1991 Cantara Spill based on visual shoreline counts expanded upwards using species category-specific expansion factors derived from Conant Experiment.

Stratum	Trout	Sucker	Pikeminnow-hardhead	Sculpin	Crayfish	Bass	Total
Reach 1	61,240	27,814	63,608	333,204	26,412	2,632	514,910
Reach 2	103,082	1,246	10,192	109,822	17,032	0	241,374
Reach 3	72,308	0	0	306,432	1,914	0	380,654
Total	236,630	29,060	73,800	749,458	45,358	2,632	1,136,938

known hatchery fish baseline sample, 4 of 47 fish were misclassified as wild fish, giving an apparent misclassification rate of approximately 9%.

When the derived discriminant function was used to classify the large sample of fish of unknown origin, 497 of 586 fish were classified as wild fish, giving an overall estimated proportion of 85% wild fish. As illustrated by Fig. 3, however, when the proportion of 1 category greatly exceeds the proportion of a 2nd category, discriminant analysis will somewhat underestimate the true numbers in the larger category and substantially overestimate the true numbers in the smaller category. In this instance this leads to a small negative bias in the resulting estimate of the proportion of wild fish and a large positive bias in the estimate of the proportion of the hatchery fish.

Application of distribution mixture separation methods (MacDonald and Pitcher 1979) to the calculated discriminant scores of fish in the sample of fish of unknown

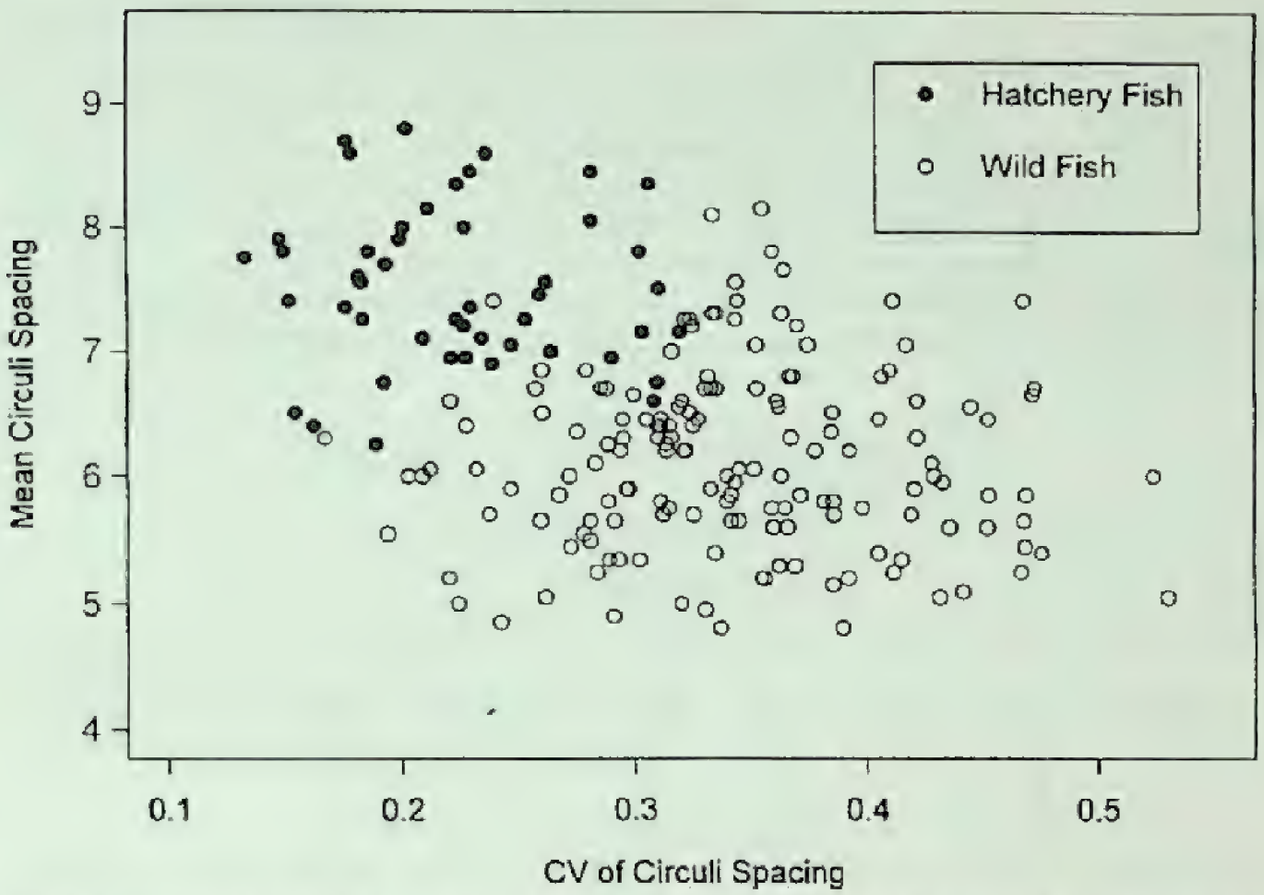

Figure 2. Mean circuli spacing (in standardized sampling units) plotted against coefficient of variation (CV) of circuli spacing based on measurements of the first 15 circuli for a known sample of hatchery rainbow trout (n = 47) similar to those stocked in the upper Sacramento River and for a presumptively known sample of wild rainbow trout (n = 170) from dipnet collections of carcasses after the Cantara Spill.

origin resulted in estimated proportions of wild fish among the Cantara trout kills that ranged from 90 to 94% for the reduced data set (n = 416, excluding those presumptive wild fish used to derive the discriminant function), and from 93 to 96% for the full data set (n = 586, including those presumptive wild fish used to derive the discriminant function), depending on analysis assumptions. Thus, the estimated number of wild fish in the full sample was 545–563, whereas hatchery fish numbered only 23–41. Chi-square goodness-of-fit statistics for all fitted distribution mixtures failed to suggest a significant lack of fit of the predicted as compared to observed frequencies of discriminant scores (Table 5).

DISCUSSION

Estimated Fish Kills

The estimated fish kills reported in this paper probably have substantial negative bias when compared to the true fish kills that must have resulted from the 1991 Cantara Spill. Good reasons to suspect negative bias include at least the following: 1) loss of dead fish due to decomposition or disintegration of carcasses prior to and during post-spill fish counting surveys; 2) inaccessibility or "invisibility" of dead fish on the bottom of very deep pools; 3) loss of carcasses carried by the current into

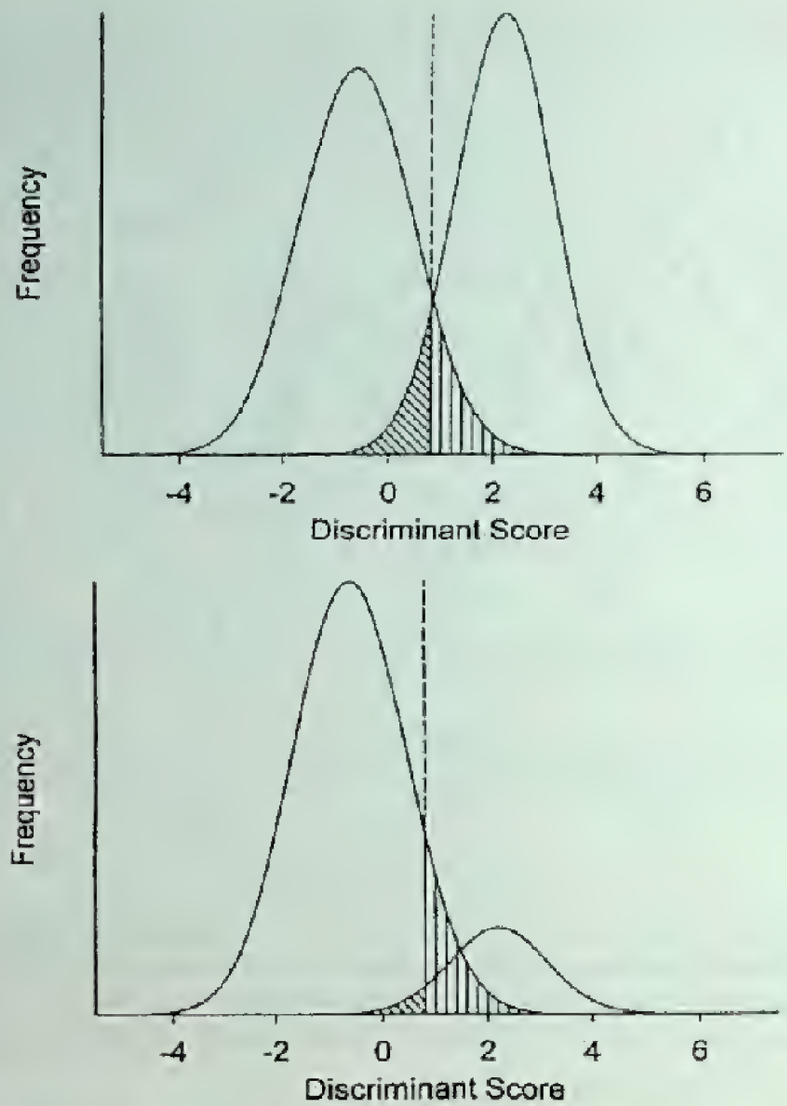

Figure 3. An illustration of numbers of individuals misclassified for a situation in which distributions of discriminant scores are normal and when the true proportions of the 2 categories are each 50% (top) or when the true proportions of the 2 categories are 85% and 15%, respectively (bottom). The vertical dashed lines indicate the cutoff value used to distinguish between the 2 categories. Means and variances for the plotted distributions are the same as the sample means and variances of calculated discriminant scores for wild rainbow trout (left distribution) and hatchery rainbow trout (right distribution) in the upper Sacramento River based on data plotted in Fig. 2. Vertical hatched region displays wild fish that would be misclassified as hatchery fish, whereas angled hatched region depicts hatchery fish that would be misclassified as wild fish.

Shasta Lake, beyond the surveyed reaches of the upper Sacramento; and, especially, 4) inability to effectively locate, count, and collect small individuals of all species. In writing about the Conant Experiment, for example, D. Hoopaugh (CDFG, unpublished memorandum) noted that "at one location hundreds, perhaps thousands, of fingerling trout were lodged in the cobble bottom of a quiet area adjacent to the upper pool. Approximately 1–2 dozen fish would float downstream whenever a rock was dislodged [, but] it was impossible to collect them ..... because the advanced state of decomposition resulted in their disintegration whenever they were touched." There is unfortunately no basis by which to judge the probable magnitude of such negative bias.

Table 5. Estimated proportions of wild and hatchery fish among dead rainbow trout collected from the upper Sacramento River following the 1991 Cantara Spill based on application of distribution mixture separation methods (MacDonald and Pitcher 1979) to calculate discriminant scores derived from scale measurements. Estimated proportions and their standard errors are denoted by appropriately subscripted $\hat{P}$ and SE ($\hat{P}$). Estimated means and standard deviations of discriminant scores are denoted by appropriately subscripted $\hat{\mu}$ and $\hat{\sigma}$. Chi-square statistic and degrees of freedom measure goodness-of-fit of the fitted distributions and estimated proportions as compared to observed frequencies of discriminant scores. "Reduced data" excludes trout ≤178 mm that were used to develop the discriminant function, whereas "All data" includes these small, presumptively wild, trout.

	Wild trout				Hatchery trout						
	$\hat{P}_w$	SE($\hat{P}_w$)	$\hat{\mu}_w$	$\hat{\sigma}_w$	$\hat{P}_h$	SE($\hat{P}_h$)	$\hat{\mu}_h$	$\hat{\sigma}_h$	df	χ^2	P
					All data (n = 586)						
Fixed μ and σ	0.930	0.013	-0.609	1.017	0.070	0.013	2.203	0.936	40	53.17	0.079
$\sigma_w = \sigma$	0.955	0.015	-0.427	1.026	0.045	0.015	2.664	1.026	37	38.85	0.386
Free search	0.960	0.020	-0.418	1.033	0.040	0.020	2.818	0.918	36	38.78	0.345
					Reduced data (n = 416)						
Fixed μ and σ	0.896	0.018	-0.609	1.017	0.104	0.018	2.203	0.936	40	46.03	0.237
$\sigma_w = \sigma$	0.933	0.021	-0.358	1.020	0.067	0.021	2.618	1.020	37	28.44	0.843
Free search	0.935	0.037	-0.353	1.023	0.065	0.037	2.670	0.988	36	28.43	0.811

Because post-spill sampling locations were not selected according to a rigorous probability survey design, and because substantial negative non-sampling bias in estimated fish kills seems likely (discussed above), we have not calculated error bounds for our estimated fish kills. Although the methods that we used to estimate fish kills were rigorous and appropriate for analysis of survey data collected by simple random sampling, the formal statistical properties of the stratified ratio estimator are unknown when units are selected purposively, as was the case for the post-spill surveys. Especially given the substantial delay between the toxic spill itself and when conditions were judged safe for field surveys, there seems little justification for failure to have adopted a rigorous, randomized, survey design for estimation of the total fish kill (see, e.g., Chapters 2 and 3 in AFS 1992).

Even if the original survey locations had been selected according to some randomized sampling design, however, the negative non-sampling bias discussed above would have frustrated meaningful interpretation of formal calculations of errors of estimation. For biased estimators, the appropriate measure of estimation error is mean square error: sampling variance plus the square of bias. Because non-sampling bias may be large and negative for estimated Cantara fish kills, any formal calculations of estimation error would probably greatly underestimate true estimation error.

Although post-spill counts and collections of dead fish were not made according to a randomized survey design, it nevertheless seems appropriate to state the positive attributes of the fish kill survey data and derived estimates of fish kills. First, survey counts were made along a substantial portion (about 14%) of the entire shoreline of the affected total stream reach, and survey locations were selected from throughout the affected stream length. Second, although locations for counts and dipnet collections were selected in part on the basis of accessibility, there is no particular reason to suspect that the post-spill distribution of dead carcasses should be greatly affected by accessibility of survey locations; dead fish were likely transported substantial distances from their original live locations. However, in surveys of live fish, purposive selection of survey locations with greater accessibility can be assumed to result in substantial, although unknown, bias. Finally, 2 quite different methods used to expand the visual counts of fish to estimates of total fish kills resulted in very similar estimated total kills: approximately 1.125 million and 1.137 million fish, respectively. There seems no question that large numbers of trout and sculpins were killed by the Cantara Spill, and it seems clear that there were important differences in the distribution of various fish species in the upper Sacramento River. The lower river (Reach 1) had greatest fish diversity, whereas the upper river (Reach 3) apparently had an impoverished community of fishes, dominated almost entirely by trout and sculpin.

Although the estimated total fish kills probably provide good minimum estimates of the total number of fish killed, the separation of total fish kill into species categories is more problematic. In the shoreline visual counts, approximately 42% of all counts were assigned to the "unidentified fish" category. These fish were allocated to identified species categories assuming that the distribution by species categories of unidentified fish was the same as that for those fish that had been identified by species category. Although this method of allocation was reasonable and without prejudice, it is conceivable that the unidentified fish category consisted primarily of nongame fish or small trout or had some other distribution by species category that was markedly different from that for identifiable fish. In principle, data collected during the Conant Experiment might have suggested alternative allocations of fish in the unidentified category. However, divers did not identify collected fish according to species category, but instead merely counted and collected fish for storage (freezing). Because numbers of fish collected by snorkeling (1,383) exceeded the number of fish collected by dipnet (1,226) in the Conant Experiment, assignment of unidentified fish to species categories was again problematic. Following future toxic spills, we recommend that several, randomly located experiments like the Conant Experiment be carried out, but that all fish should be identified to species categories during the experiment itself; identification after the fact from a mass of decomposing frozen material is extremely difficult and may never take place.

Estimated Proportion of Wild Fish

Results from discriminant analysis and subsequent application of distribution mixture separation methods to calculated discriminant scores suggest that wild fish

constituted from 90 to 96% of the rainbow trout present at the time of the spill. These high estimated proportions of wild fish are similar to Nielsen et al.'s (2000) estimate, using genetic methods, that 83% of the rainbow trout killed by the spill were genetically different from Mount Shasta Hatchery trout. The lower value estimated by Nielsen et al. (2000) may reflect a modest amount of interbreeding between wild and hatchery fish; such fish would probably be classified as hatchery fish based on genetic methods, whereas they would be classified as wild fish (i.e., the result of natural spawning and rearing in the upper Sacramento River) by methods used in this paper. These 2 estimates may be compared to the pre-spill estimate by Rode and Zuspan[2] (1993), based on coloration, body form, and fin abrasion, that about 90% of trout collected in 1980–1981 were of wild origin. As for the estimated kills of fish resulting from the Cantara Spill, however, the above estimates of wild fish proportions treat sample data as if they were drawn as a single simple random sample from the total population of rainbow trout in the upper Sacramento River. In truth, samples originated from a variety of locations, sample sizes at locations varied, and there was no clear probability structure to selection of those locations where samples were collected. Thus, all of these estimates lack the underlying structure of formal probability samples and results from analysis of a combined sample may be misleading. Nevertheless, the essential strong agreement of estimated proportions of wild fish in these 3 studies provides substantial support for a contention that the highly regarded rainbow trout fishery in the upper Sacramento River has been dominated by wild trout.

In recognition of the importance of wild trout to the upper Sacramento River's blue ribbon fishery for rainbow trout, sport fishery regulations adopted following the 1991 Cantara spill have further emphasized wild fish. When the fishery was first reopened in April 1994, approximately 52 km of river in 2 sections (the upper 10 km and the lower 42 km) were opened to catch and release angling only, whereas the harvest fishery (5 fish per day) was limited to the intervening 10-km stretch where hatchery fish were stocked.

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MOLECULAR ANALYSIS OF POPULATION GENETIC STRUCTURE AND RECOLONIZATION OF RAINBOW TROUT FOLLOWING THE CANTARA SPILL

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Mitochondrial DNA (mtDNA) sequence and allelic frequency data for 12 microsatellite loci were used to analyze population genetic structure and recolonization by rainbow trout, *Oncorhynchus mykiss*, following the 1991 Cantara spill on the upper Sacramento River, California. Genetic analyses were performed on 1,016 wild rainbow trout collected between 1993 and 1996 from the mainstem and in 8 tributaries. Wild trout genotypes were compared to genotypes for 79 Mount Shasta Hatchery rainbow trout. No genetic heterogeneity was found 2 years after the spill (1993) between tributary populations and geographically proximate mainstem fish, suggesting recolonization of the upper mainstem directly from adjacent tributaries. Trout collections made in 1996 showed significant year-class genetic variation for mtDNA and microsatellites when compared to fish from the same locations in 1993. Five years after the spill, mainstem populations appeared genetically mixed with no significant allelic frequency differences between mainstem populations and geographically proximate tributary trout. In our 1996 samples, we found no significant genetic differences due to season of capture (summer or fall) or sampling technique used to capture rainbow trout, with the exception of trout collected by electrofishing and hook and line near Prospect Avenue. Haplotype and allelic frequencies in wild rainbow trout populations captured in the upper Sacramento River

and its tributaries were found to differ genetically from Mount Shasta Hatchery trout for both years, with the notable exception of trout collected in the lower mainstem river near Shasta Lake, where mtDNA and microsatellite data both suggested upstream colonization by hatchery fish from the reservoir. These data suggest that the chemical spill in the upper Sacramento River produced significant effects over time on the genetic population structure of rainbow trout throughout the entire upper river basin.

INTRODUCTION

On the night of 14 July 1991, a tanker car filled with the pesticide metam sodium (sodium N-methyl dithicarbamate) derailed and overturned into the upper Sacramento River at the Cantara Loop (Fig. 1). The toxic plume from the contents of this car (72,000 liters) devastated 58 km of one of California's premier trout streams, killing virtually all downstream aquatic life including approximately 300,000 rainbow trout, *Oncorhynchus mykiss* (Hankin and McCanne 2000). Before the spill, the mainstem Sacramento River near the town of Dunsmuir supported a popular recreational trout fishery supplemented by stocking large numbers (60,000–94,000) of Mount Shasta Hatchery rainbow trout annually from 1980 to 1990. The lower 21 km of the upper mainstem upstream from Shasta Lake, however, have not been stocked with hatchery fish since 1982 and contained a fish assemblage dominated by trophy-sized wild rainbow trout (Paine and Associates[1] 1995).

Electrofishing surveys (1980–1981) in the upper Sacramento River 1 year after the release of tagged hatchery rainbow trout estimated that hatchery fish represented 5–17% of the trout present in the mainstem upper Sacramento River, suggesting limited survival of hatchery stocks in the wild (Rode[2] 1994). Discriminate analysis of scale circuli patterns from trout carcasses collected after the Cantara spill conservatively estimated that 7–16% of the surveyed trout (n = 586) could be assigned to hatchery trout circuli patterns (Hankin and McCanne 2000). Mitochondrial DNA sequence data showed that 83% of the dead trout analyzed after the Cantara fish kill (n = 60) were genetically distinct from the Mount Shasta Hatchery rainbow trout strain stocked in the Sacramento River (Nielsen et al.[3] 1996).

Shortly after the Cantara spill, the California Department of Fish and Game (CDFG) closed the upper Sacramento River and all of its tributaries to sport fishing to allow the river ecosystem to recover and no hatchery trout were introduced for

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[1] Payne, T.R. and Associates. 1995. Recovery of fish populations in the upper Sacramento River following the 1991 Cantara spill. Unpublished report submitted to Cantara Project, California Department of Fish and Game, Redding, California, USA.

[2] Rode, M. 1994. Upper Sacramento River fishery investigations, part I: Fish populations, migration, angler harvest, and water temperature studies. Region 1, California Department of Fish and Game, Redding, California, USA.

[3] Nielsen, J.L., C.A. Gan, and M.C. Fountain. 1996. Molecular analysis of population structure and recolonization trends in rainbow trout following a fish kill event. Technical report submitted to the Cantara Program, California Department of Fish and Game, Redding, California, USA.

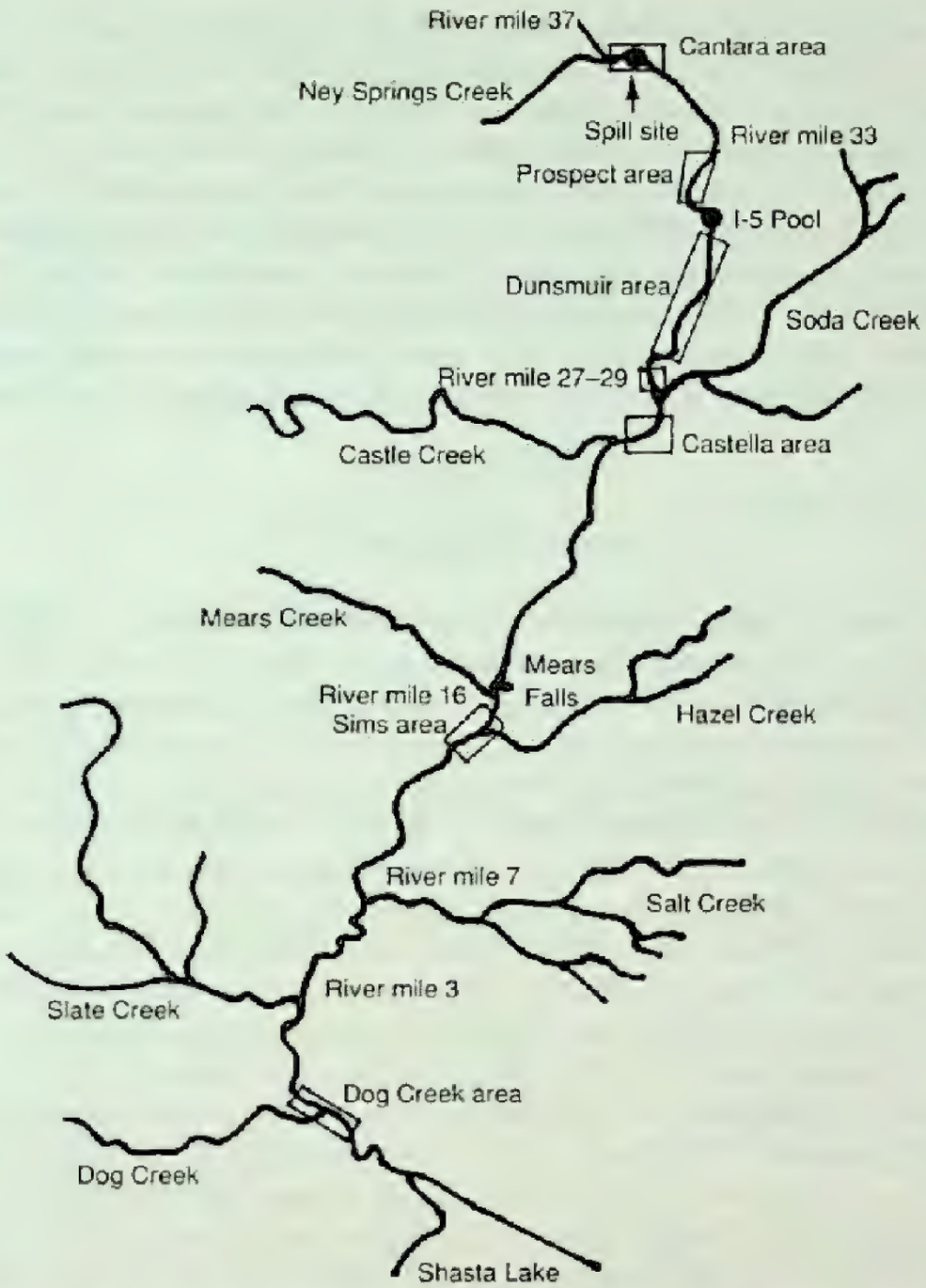

Figure 1. Map showing the general sampling locations used to collect upper Sacramento River rainbow trout in 1993 and 1996 for this study.

3 years (1991–1994). To date, the recovery of wild trout in the mainstem following the spill has been very successful (Turek[4] 1998). Stocking of Mount Shasta Hatchery rainbow trout began again in 1994 (approximately 16,000/year) in the Dunsmuir area of the mainstem upper Sacramento River (Turek[4] 1998).

With a high probability of limited recruitment from hatchery stocks over the last 20 years and a large proportion of mainstem wild trout destroyed by the Cantara

[4] Turek, S. 1998. The use of angler surveys in managing the recovery of the upper Sacramento River wild trout fishery. Unpublished technical report submitted to the Cantara Trustee Council, February 1998. California Department of Fish and Game, Redding, California, USA.

spill, some important questions arose. How were the current trout numbers recruited to the mainstem habitats? What type of genetic population structure can evolve over the short term following a fish-kill event? What type of management strategy should be in place to provide fish for both a recreational fishery and the conservation of the unique wild trout gene pool in the upper Sacramento River? In this study, we analyzed genetic diversity based on mtDNA and 12 microsatellite loci in 8 tributary trout populations and geographically proximate mainstem populations, first in 1993 and again in 1996. We looked for patterns of colonization in the mainstem river 3 and 5 years after the fish-kill event and tested the genetic effects of recent supplementation by hatchery fish in the upper Sacramento River on the recovered wild trout population.

METHODS

Tissue Collections

Dried fin tissues (2 mm^2) were collected by noninvasive means in hatchery and wild rainbow trout populations from throughout the upper Sacramento River and from the Mount Shasta Hatchery according to methods given in Nielsen et al. (1997a). Dried fin clips were collected from 1,016 rainbow trout in 8 tributary and 11 mainstem locations in the upper Sacramento River drainage by the CDFG using electrofishing and volunteer angler hook-and-line surveys (Table 1). Mainstem samples collected in 1993 were electrofished from 1 site at each river location. Mainstem sample areas pooled for 1996 data analysis due to small sample size included: 1) "Cantara area" = 31 fish from 2 collections (September and November) in the Cantara area (no site locations listed); 2) "Prospect area" = 4 fish from river miles (rm) 31 and 32 (river km [rkm] 50 and 52) and 60 fish from the mainstem at Prospect Avenue; 3) "Dunsmuir area" = 6 fish caught at Financial Avenue, 8 fish caught at South 1st Street, 18 fish from the mainstem at Dunsmuir (no site locations listed), 3 fish from Shearer 1st Bridge, and 2 fish caught at River Avenue; 4) "rm 27–29" = 4 fish from rm 27 (rkm 43), 2 fish from rm 28 (rkm 45), and 3 fish from rm 29 (rkm 47); 5) "Castella area" = 8 fish from rm 26 (rkm 42), 13 fish from Castella Camp, and 34 fish from the Castella area (no site locations listed); 6) "Sims area" = 33 fish from Sims area (no site locations listed); and 7) "Dog Creek area" = 32 fish from Dog Creek area of the mainstem river (no site locations listed) (Fig. 1).

To test for collection-method bias, in 1996 we used trout fin-clips collected by electrofishing and hook-and-line samples collected at different times of the year (summer and fall) in 5 locations: Prospect area, Castella area, Sims area, Dog Creek area, and Soda Creek.

Mitochondrial DNA

We extracted total genomic DNA from trout fin tissues using an insoluble chelex resin containing paired iminodiacetate ions that chelate polyvalent metal ions (Walsh et al. 1991) following methods given in Nielsen et al. (1994b). We amplified and

Table 1. Sampling locations and number of fish analyzed for each molecular locus used in this study. Sample gears were electrofishing (EL) and hook and line (HL). N = sample sizes for 1993 (3 loci) and 1996 (12 loci). Rm = river mile.

Sample	Year(N)	Sample gear	mtDNA	Locus											
				Oneu2	Oneu8	Oneu11	Oneu14	Omy2	Omy27	Omy77	Omy325	Ots1	Ssa14	Ssa85	Ssa289
Mainstem Sacramento River															
Rm 37	93(28)	EL	28	0	0	0	0	22	12	18	0	0	0	0	0
Cantara area	96(31)	HL	10	30	30	31	27	0	31	29	31	25	22	29	31
Prospect area	93(29), 96(60)	HL, EL	36	64	61	62	61	29	72	89	64	62	28	56	62
I-5 pool	96(30)	EL	9	30	24	29	30	0	30	30	27	28	24	29	28
Dunsmuir area	96(71)	HL, EL	12	67	61	67	63	0	64	61	64	66	59	63	66
Rm 27–29	93(29), 96(30)	HL, EL	37	9	9	9	9	29	21	39	9	9	9	9	9
Castella area	96(85)	HL, EL	12	85	83	85	82	0	85	84	83	81	67	81	85
Sims area	93(29),96(65)	HL, EL	15	60	63	62	60	8	68	92	62	59	57	62	60
Rm 3	93(26), 96(27)	EL	11	1	1	1	1	10	10	27	0	1	1	1	1
Dog Creek area	96(32)	HL	10	32	29	32	29	0	32	29	29	29	23	31	32
Sacramento River Tributaries															
Ney Springs Creek	93(27), 96(60)	EL	40	60	59	55	59	30	70	88	61	56	44	59	60
Soda Creek	93(28), 96(30)	HL, EL	42	43	43	45	44	27	54	54	43	43	40	45	43
Castle Creek	96(9)	EL	8	9	9	9	9	0	9	9	9	9	6	9	8
Mears Creek	93(30)	EL	30	13	11	9	12	30	17	32	11	13	10	12	13
Hazel Creek	96(31)	EL	8	30	31	31	29	0	24	31	26	29	9	29	11
Salt Creek	93(29)	EL	7	5	6	9	2	9	6	29	8	10	10	8	7
Slate Creek	93(30)	EL	21	7	8	10	6	26	14	30	8	7	10	8	10
Dog Creek	93(30), 96(61)	EL	38	58	51	61	56	29	69	86	55	58	50	59	61
Mt. Shasta Hatchery	93(40), 96(39)	EL	73	35	35	35	27	21	52	75	35	35	22	35	35
Total counts	1,016		447	638	614	642	606	270	740	932	625	620	491	625	622

sequenced 188 bases of the highly variable mtDNA control region and 5 base pairs of the adjacent phenylalanine tRNA gene using conserved primers and standard polymerase chain reaction (PCR) protocols. Mitochondrial DNA control-region primers (Sphe and P2), the complete sequence for rainbow trout in this region, and our sequence amplification protocols were given in Nielsen et al. (1994a). Polymerase chain reaction products were sequenced directly and the DNA visualized on x-ray film. Sequences were aligned using MacDNASIS (Hatachi America, Ltd.[5], San Francisco, California, USA, 1991). To insure consistency in both the PCR reactions and the scoring of the mtDNA, 2.4% of all samples were rerun separately on different gels and scored independently.

Nomenclature for mtDNA control region haplotypes in *O. mykiss* follows those given in Nielsen et al. (1997b). We used an unbiased estimate of the Fisher's exact test from GENEPOP (Raymond and Rousset[6] 1995), based on a Markov chain adaptation of row-by-column contingency tables (Guo and Thompson 1992), to test for differentiation in haplotype frequencies between all possible paired populations of rainbow trout.

Microsatellites

Twelve microsatellite loci were chosen for these analyses based on their high level of polymorphism in previous studies of rainbow trout and steelhead (anadromous form of *O. mykiss*) in our laboratory. The Omy-series of microsatellite loci was developed specifically for rainbow trout; the Oneµ-series was developed for sockeye salmon, *Oncorhynchus nerka*; the Ots-series was developed for chinook salmon, *O. tshawytscha*; and the Ssa-series was developed for Atlantic salmon, *Salmo salar*. Amplification of microsatellites followed methods in Nielsen et al. (1997b), except that each 7.15-µliter PCR reaction contained 67 µM Tris-HCl (pH 8.8); 6.7 µM $MgCl_2$; 16.6 µM $(NH_4)_2SO_4$; 10 µM ß-mercaptoethanol; 1 µM each of dGTP, dATP, dTTP, and dCTP; 1 µM of each primer; 0.15 units of *Taq* polymerase; and 1 µliter of Chelex 100- (BioRad[5], Richmond, California, USA) extracted DNA. Polymerase chain reaction conditions and the color of the fluorescently labeled reverse primer were maximized for joint amplification on the gel (Table 2). Microsatellites were run out on a 6% polyacrylamide gel. Prior to loading the gel, 1 µliter PCR product was added to 4 µliter of loading buffer containing 1 µliter 50 mg/ml Blue Dextran, 2.5 µliter diformamide, and 0.5 µliter ABI Genescan 500 internal size standard (P-E Biosystems[5], Foster City, California, USA).

All microsatellite gels were run on an ABI 373 adapted for microsatellite analysis. GENESCAN version 1.1 and GENOTYPER version 2.1 (P-E Biosystems[5], Foster City, California, USA) DNA fragment analysis software packages were used to score, bin, and output allelic (and genotypic) designations. All loci were run individually

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[5] Use of trade names does not imply endorsement by the California Department of Fish and Game.

[6] Raymond, M. and F. Rousset. 1995. GENEPOP version 2.0 (July 1995). University of Montpellier II, 34095 Montpellier cedex 05, France. (e-mail: Raymond@univ-montp2.fr).

Table 2. PCR conditions used for amplification of 12 microsatellite loci from upper Sacramento River rainbow trout. Primer concentrations for all reactions were 1 M. PCRs run at the same conditions were multiplexed on the ABI sequencer gel.

	Locus		
Annealing temperature/cycles	6Fam-blue	Tet-green	Hex-yellow
52°C/30	Oneμ14	Ots1	Oneμ11
	Omy27	Ssa85	Ssa289
52°C/32	Omy77	Ssa14	Omy325
	Oneμ2		Oneμ8
50°C/30		Omy2	

in separate PCR reactions to determine the maximum allelic size distributions for Sacramento River rainbow trout. Microsatellite allele sizes (including the amplified primer) were determined in relation to the Genescan-500 internal size standard, *O. mykiss* DNA samples of known size that were rerun on each gel, and a double-stranded reference marker developed in our laboratory showing the most common alleles for each locus. To insure consistency in both PCR reactions and scoring of microsatellites, 5.9% of all samples were rerun on different gels and scored independently.

Tests for population differentiation using microsatellites were performed with GENEPOP. Fisher's exact tests using microsatellite allelic frequency data were run on all possible pairs of populations for each locus and for all loci combined. Statistical significance levels (initial $\alpha = 0.025$) for Fisher's exact analyses were set using sequential Bonferroni tests (Rice 1989). GENEPOP's Fisher's exact tests give equivalent probability values to the iterative chi-square probability test (Roff and Bentzen 1989), but have been shown to have several computational advantages in multi-loci analyses (Raymond and Rousset 1995).

Pairwise genetic distance matrices were calculated using both R_{st} (Slatkin 1995) and $\delta\mu^2$ (Goldstein et al. 1995) for 3 (1993: Omy2, Omy27, Omy77) and 11 microsatellite loci (1996: Oneμ2, Oneμ8, Oneμ11, Oneμ14, Omy27, Omy77, Omy325, Ots1, Ssa14, Ssa85, Ssa289) combined using MICROSAT 1.40[7]. Both genetic distance measures assume a linear expectation of the average squared distance for each locus (assuming no correlation between mutation rate and repeat score) and use the arithmetic average of mutation rates across loci. These statistics are equivalent to a general analysis of variance using an average sum of squares of differences in allelic size within each population and the average squared difference between all possible pairs of populations. These values are used to obtain an estimate of variance in allele size in the total population.

[7] Minch, E. Department of Genetics, Stanford University. Available at http://crick.stanford.edu/microsat/microsat.html.

Slatkin's R_{st} as performed by MICROSAT uses a ratio of combinations of the mean squared distance, which cancels out all mutation parameters in the calculation of genetic distance (Slatkin 1991). Delta μ^2 maintains an estimate of the mutation process under expectation of a strict, single-step (± 1 repeat unit) shift for each mutation event. We compared distance estimates for both years using both methods. We calculated F_{st} (fixation index; Weir and Cockerham 1984) and mean heterozygosity for the 12 microsatellite loci using MICROSAT with expected equilibrium values developed for the stepwise mutation processes (Rousset 1996).

Distance data were used to generate unrooted consensus neighbor-joining trees using the NEIGHBOR and CONSENSE applications from PHYLIP (Felsenstein[8] 1993) comparing microsatellite diversity among the upper Sacramento River rainbow trout populations. For $\delta\mu^2$ data, 1,000 replicate microsatellite distance trees were generated to obtain bootstrap estimates based on locus removal with replacement available in the $\delta\mu^2$ program. These bootstrap values were used to assess reproducibility of branching patterns found in our $\delta\mu^2$ consensus tree.

RESULTS

Mitochondrial DNA

Seven mtDNA haplotypes were found in the rainbow trout from the upper Sacramento River in 1993 and 1996 (Table 3). The MYS1 haplotype dominated most tributary populations in 1993, with the exception of Mears and Slate creeks, where only fish carrying haplotype MYS3 were found. In 1993, mtDNA haplotype MYS3 dominated upper Sacramento River mainstem rainbow trout populations,

Table 3. Mitochondrial control region variable sites and nucleotide changes (underlined bold) found in relation to MYS1 in upper Sacramento River rainbow trout sampled in 1993 and 1996. Base pair numbers are given as referenced in 2 published papers. Nucleotides are G = guanine, A = adenine, T = thymine, and C = cytosine.

	Base pair number							
mtDNA	65	88	96	149	154	194	196	(Nielsen et al. 1994a)
type	1021	1044	1052	1104	1109	1147	1149	(Digby et al. 1992)
MYS1	T	G	T	A	G	G	C	
MYS3	T	G	T	A	**A**	G	C	
MYS9	T	G	T	A	G	**A**	C	
MYS10	T	G	**C**	A	**A**	G	C	
MYS11	T	**A**	T	A	G	G	C	
MYS14	**C**	G	T	A	**A**	G	C	
MYS16	T	G	T	**G**	G	G	**T**	

[8] Felsenstein, J. 1993. PHYLIP version 3.5 (phylogeny inference package distributed by author). Department of Genetics SK-10, University of Washington, Seattle, Washington, USA.

with the exception of the lower mainstem (Sims area and rm 3), where haplotype MYS1 was more common (Table 4). Genetic population structure in this basin following the Cantara spill (1993) supported genetic differences in trout haplotype frequencies (Fisher's $P < 0.025$) in comparisons made among the following tributary populations: Ney Springs, Soda, Mears, Slate, and Dog creeks, and among trout from the 1993 mainstem sampling locations at rm 37 (rkm 60), rm 33 (rkm 53)

Table 4. Number of mtDNA haplotypes found in California rainbow trout from the upper Sacramento River in 1993 and 1996. Rm = river mile.

	mtDNA haplotype							
Population	MYS1	MYS3	MYS9	MYS10	MYS11	MYS14	MYS16	Total
Mainstem 1993								
Rm 37–Ney Springs	7	14	6	1	0	0	0	28
Prospect area	8	15	3	2	0	0	0	28
Rm 27–Soda Creek	10	17	2	0	0	0	0	29
Sims area	4	2	1	0	0	0	0	7
Rm 3	7	3	0	0	0	0	0	10
Mainstem 1996								
Cantara area	1	7	2	0	0	0	0	10
Prospect area	4	2	2	0	0	0	0	8
I-5 Pool	0	7	2	0	0	0	0	9
Dunsmuir area	3	5	4	0	0	0	0	12
Rm 27–29	3	2	3	0	0	0	0	8
Castella area	1	10	1	0	0	0	0	12
Sims area	1	6	1	0	0	0	0	8
Rm 7–Salt Creek	0	2	1	0	0	0	1	4
Rm 3	0	0	0	0	0	0	1	1
Dog Creek area	0	9	0	0	0	1	0	10
Subtotal – mainstem	49	101	28	3	0	1	2	184
Tributary 1993								
Ney Springs 1993	15	9	4	2	0	0	0	30
Soda Creek 1993	16	10	3	0	0	0	0	29
Mears Creek 1993	0	30	0	0	0	0	0	30
Salt Creek 1993	4	3	0	0	0	0	0	7
Slate Creek 1993	0	21	0	0	0	0	0	21
Dog Creek 1993	13	11	5	0	0	1	0	30
Tributary 1996								
Ney Springs 1996	1	6	2	0	1	0	0	10
Soda Creek 1996	1	9	2	0	0	0	1	13
Castle Creek 1996	1	5	2	0	0	0	0	8
Hazel Creek 1996	2	6	0	0	0	0	0	8
Dog Creek 1996	1	5	1	0	0	0	1	8
Subtotal – tributary	54	115	19	2	1	1	2	194
Mt. Shasta Hatchery								
1993	52	0	0	0	0	0	0	52
1996	21	0	0	0	0	0	0	21
Subtotal – hatchery	73	0	0	0	0	0	0	73

(near Prospect area), rm 27, rm 16 (rkm 26) (near Sims area), and rm 3 (rkm 5). Tributary and geographically adjacent mainstem trout populations in 1993 were significantly different ($P = 0.017$) for mtDNA haplotype frequencies only in our comparison of Mears Creek and the Sims area.

Mitochondrial DNA frequency distributions were significantly different in 1996 when compared to 1993 samples taken in the same localities (Fisher's $P < 0.025$ in all comparisons). MYS3 increased in frequency in tributary populations in our 1996 collection (Table 4). The 1996 mainstem trout populations remained dominated by MYS3, with the exception of several populations collected in the vicinity of Dunsmuir (rm 27, Financial Avenue, Prospect Avenue), where MYS1 was found to be equally common. We found no significant haplotype differentiation among tributary or mainstem populations in 1996 (Fisher's $P > 0.12$ in all comparisons). Tributary and adjacent mainstem collection sites were also found to lack significant mtDNA differentiation in 1996 (Fisher's $P > 0.19$ in all comparisons).

Two rare haplotypes were found in individual trout collected in Ney Springs Creek (1996, MYS11), Dog Creek (1993, MYS14), and the mainstem area near Dog Creek (1996, MYS14). In 1996, a unique haplotype, MYS16, previously found only in volcano golden trout, *Oncorhynchus mykiss aquabonita*, and Eagle Lake rainbow trout, *O. m. aquilarum*, was found in 2 fish collected in the lower mainstem river and in 1 fish from Soda Creek.

In both years, mtDNA haplotype MYS1 was monomorphic in Mount Shasta Hatchery rainbow trout. Significant differences occurred between Mount Shasta Hatchery mtDNA haplotype frequencies (monomorphic for MYS1) and all upper Sacramento River wild trout populations (Fisher's $P < 0.0001$ in both years), with the exception of fish collected at rm 3 (1993 $P = 0.026$). Haplotype MYS16, found at rm 3 in 1996, has never been recorded in Mount Shasta Hatchery rainbow trout. A rare haplotype, MYS10, found in several California rainbow trout hatchery strains, but not in the Mount Shasta strain (see Nielsen et al.[3] 1996), was found in Ney Springs Creek, adjacent upper mainstem Sacramento River, and the Prospect area in 1993.

Microsatellites

The 12 microsatellite loci used to test genetic population structure and recolonization trends in upper Sacramento River rainbow trout were highly polymorphic (Table 5). The number of alleles ranged from 4 (Oneμ11) to 30 (Oneμ2), and allelic sizes ranged from 89 bases (Omy325) to 280 bases (Oneμ2). Mean standard deviation for GENOTYPER size estimates of alleles for all loci combined was 0.26 base pairs. Average heterozygosity for all microsatellite loci combined was 0.68 in both 1993 and 1996 (range 0.46 [Omy2]–0.81 [Oneμ2]). Average F_{st} was 0.048 (1993) and 0.018 (1996), with a 1996 range from 0.01 (Ots1) to 0.06 (Ssa85).

Recent studies have shown null alleles (Omy77 and Oneμ14) and size homoplasy (Oneμ11) in a bottlenecked population of *O. mykiss* from Alaska (Ardren et al. 1999). We ran $\delta\mu^2$ genetic distance analyses on the upper Sacramento River rainbow

Table 5. List of 12 microsatellites and their source publications amplified from upper Sacramento River rainbow trout sampled in 1993 and 1996. Size SD is the standard deviation calculated for allelic size estimates made at each allele for each locus amplified from upper Sacramento River rainbow trout.

Locus	Source	Number alleles	Allelic size (bp)	Size SD (bp)
Omy2	M. O'Connell[a]	21	108–166	0.21
Omy27	M. O'Connell[a]	7	99–117	0.24
Omy77	Morris et al. 1996	20	97–141	0.26
Omy325	M. O'Connell[a]	22	89–139	0.36
Onep2	Scribner et al. 1996	30	200–280	0.22
Onep8	Scribner et al. 1996	17	148–190	0.36
Onep11	Scribner et al. 1996	4	145–151	0.17
Onep14	Scribner et al. 1996	13	147–179	0.20
Ots1	M. Banks[b]	17	155–241	0.24
Ssa14	McConnell et al. 1995	16	126–158	0.21
Ssa85	O'Reilly et al. 1996	25	95–157	0.38
Ssa289	McConnell et al. 1995	12	100–126	0.26

[a] M. O'Connell, Guelph University, City, Ontario, Canada, personal communication.
[b] M. Banks, University of California, Davis, California, USA, personal communication.

trout without each of these loci and without all 3 loci combined to analyze the relative contribution of each locus on our overall findings. These analyses did not change the architecture of the genetic distance associations found among the upper Sacramento River rainbow trout populations collected in 1993 or 1996. Variation found at each locus acted on all populations with relatively equal effect.

The removal of each locus individually or all 3 loci combined caused an increase in the estimated genetic distances calculated for all loci combined (i.e., a change in branch lengths but not tree structure), suggesting that in this analysis these loci dampened overall distance estimates. Delta μ^2 values calculated without Omy77 increased by 4% on average; without Onep11, they increased by 8% on average; and without Onep14, they increased 7% on average. When all 3 loci were removed from the analysis at the same time, $\delta\mu^2$ values increased 32% on average (range 25–36%).

Analyses of genetic differentiation among trout populations 2 years after the Cantara spill using 3 microsatellite loci (Omy2, Omy27, and Omy77) demonstrated a lack of significant separation between Ney Springs and Soda creeks and geographically proximate upper Sacramento River rainbow trout populations (rm 27 and rm 33; Fisher's combined $P > 0.03$ in all cases). Genetic distance (R_{ST}) analysis of the 1993 trout populations showed geographic continuity among sampling locations found in upper and lower sections of the drainage divided by Mears Falls with the exception of Mears and Slate creeks, tributaries south of the falls that were combined in this analysis with northern mainstem sites (Fig. 2).

Rainbow trout populations in 2 southern tributaries (Salt and Dog creeks) and the southernmost mainstem shared limited genetic associations with the Mount

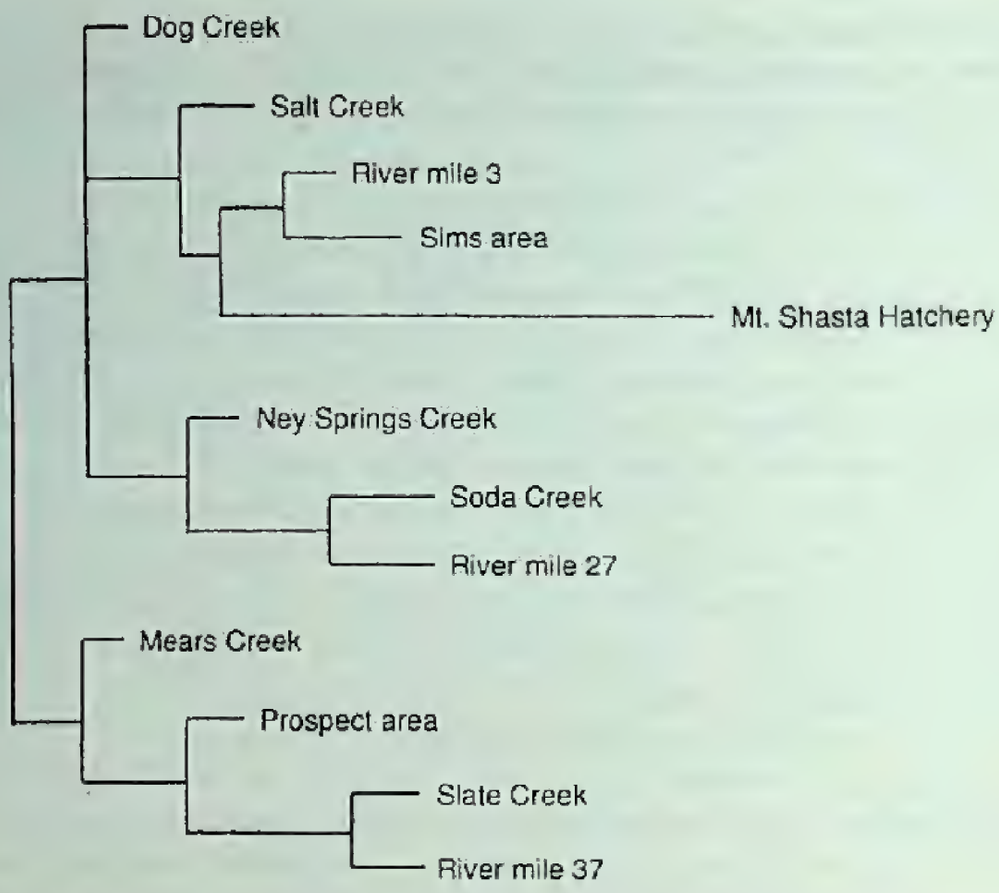

Figure 2. Genetic distance analyses calculated using Slatkin (1995) R_{st} values derived from the 1993 rainbow trout populations collected from the upper Sacramento River basin.

Shasta Hatchery trout in 1993, but R_{st} values between hatchery trout and all wild populations remained high (mean $R_{st} = 0.15$). Delta μ^2 analysis of the 1993 data gave poor geographic associations among tributary and mainstem populations (see Nielsen et al.[3] 1996 for data). With our $\delta\mu^2$ analyses, weak bootstrap values (38–69%) separated all tributary and mainstem rainbow trout populations. The 1993 $\delta\mu^2$ genetic distance analysis did support significant separation between Mount Shasta Hatchery fish and all 1993 upper Sacramento River wild trout populations with a bootstrap value of 96%.

We found significant variation between 1993 and 1996 for all microsatellite loci amplified from wild trout captured in the same tributaries and upper mainstem locations in both years. Tests of genetic differences for all sites and loci combined showed significant differences between collections made at the same site in each of the 2 years (Fisher's exact $P < 0.0001$).

Rainbow trout collected in 1996 showed limited geographic associations among sampling sites. Tests for genetic differences among 1996 trout populations for all loci combined were not significant in comparisons of all tributary populations (Fisher's exact $P = 0.22$), all mainstem sites ($P = 0.24$), and comparisons of tributary populations with geographically proximate mainstem sites ($P = 0.25$). These data suggest a lack of genetic differentiation in wild rainbow trout populations at any geographic scale in the upper Sacramento River in 1996. The only sampling location that showed significant differences in microsatellite allelic frequencies similar to

our 1993 collection was Ney Springs Creek, where 5–9 loci showed significant genetic differences in comparisons with Castle, Soda, Hazel, and Dog creeks (P <0.02 in all comparisons).

Delta μ^2 1996 genetic distance values ranged from 0.318 (Prospect area and Castella area) to 25.618 (Cantara area and Mount Shasta Hatchery trout) (Table 6). Neighbor-joining analysis calculated from $\delta\mu^2$ values showed no continuity between geographically proximate tributary and mainstem sites in the upper Sacramento River in 1996 (Fig. 3). This analysis did support a separation between 1996 Dog Creek trout and all other wild trout populations with a bootstrap value of 99%.

Wild trout caught by hook and line in the Prospect area in June and July were significantly different genetically from electrofished trout taken at the same location in November (Fisher's $P = 0.01$). Thirty fish were sampled by each method, and average fish size was similar between the collections (electrofish mean size = 237.8 mm, SE = 10.9 mm; hook-and-line mean size = 217.8 mm, SE = 13.7 mm). Delta μ^2 genetic distance values calculated between the Prospect area mainstem trout and the Mount Shasta Hatchery trout for hook-and-line samples was 13.45. The $\delta\mu^2$ value calculated for the 1996 electrofished sample at the same locations was 22.56. At all other sites, no significant genetic differences between sampling methods or time-of-year were found (P >0.38 in all comparisons).

Similarities in allelic frequencies for 1 of the 12 microsatellite loci (Ots1) were found between hatchery trout and upper mainstem trout populations near Dunsmuir in 1996, where Fisher's P values ranged from 0.24 (I-5 pool and Shasta Hatchery) to 0.5 (Financial Avenue and Shasta Hatchery). Fisher's combined comparisons for all loci, however, did not support this single-locus association. Our analysis of all wild rainbow trout populations captured in the upper Sacramento River in both years found significant genetic differences when compared to Mount Shasta Hatchery trout (Fisher's P <0.001 in all cases), with the notable exception of trout collected in Dog Creek (Fisher's 1993 $P = 0.14$, 1996 $P = 0.089$) and the lower mainstem river near Shasta Lake (1993 $P = 0.124$, 1996 $P = 0.098$).

Delta μ^2 genetic distance measures calculated for comparisons between wild trout and Mount Shasta Hatchery fish in 1996 ranged from 8.78 (Dog Creek and Mount Shasta Hatchery) to 25.62 (Cantara mainstem and Mount Shasta Hatchery). The locus Oneμ2 proved to be a good diagnostic locus for hatchery versus wild trout comparisons. Mount Shasta hatchery fish shared only 3 of the 26 alleles found in wild trout in the upper Sacramento River at this locus. Eleven alleles found in over 5% of the wild upper Sacramento River rainbow trout, but not found in any of the Mount Shasta Hatchery rainbow trout (1993 and 1996), were identified in 6 loci (Oneμ2 [n = 2], Oneμ8 [n = 2], Oneμ14 [n = 1], Omy325 [n = 3], Ssa14 [n = 2], Ssa289 [n = 1]).

DISCUSSION

Tributary habitats adjacent to the mainstem following the spill could have contained trout that migrated up the tributaries from the mainstem Sacramento River to find fresh water and avoid the spill (S. Turek, CDFG, personal communication;

Table 6. Genetic distance measures for 3 microsatellite loci (above the diagonal) using Slatkin's (1995) R_{st} (1993 trout populations) and for 11 microsatellite loci (below the diagonal) using Goldstein et al.'s (1995) $\delta\mu^2$ (all 1996 trout populations and a subset of 1993 fish) (see Table 1) amplified from rainbow trout captured in the Upper Sacramento River and the Mount Shasta Hatchery. Not all populations were sampled in both years (year listed when only 1 year sampled) and only populations with ≥9 individuals with microsatellite data were included in genetic distance analyses. "Areas" are general mainstem sampling areas (see Methods). na = not analyzed.

Population (year)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. River mile 37 (93)	–	na	0.016	na	na	0.046	na	0.252	0.169	na	0.016	0.030	na	0.039	na	0.007	0.150	0.025	0.043
2. Cantara area (96)	na	–	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
3. Prospect area	na	1.581	–	na	na	0.004	na	0.218	0.134	na	0.004	0.001	na	0.007	na	0.023	0.131	0.005	0.010
4. I-5 Pool (96)	na	2.652	0.320	–	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
5. Dunsmuir area (96)	na	0.786	0.500	0.904	–	na	na	na	na	na	na	na	na	na	na	na	na	na	na
6. River mile 27–29	na	1.722	1.216	1.734	0.988	–	na	0.234	0.154	na	0.002	0.000	na	0.001	na	0.045	0.162	0.009	0.015
7. Castella area (96)	na	1.907	0.318	0.484	0.960	0.870	–	na	na	na	na	na	na	na	na	na	na	na	na
8. Sims area	na	1.356	0.603	1.546	0.625	0.351	0.589	–	0.037	na	0.140	0.174	na	0.064	na	0.277	0.067	0.193	0.063
9. River mile 3 (93)	na	na	na	na	na	na	na	na	–	na	0.063	0.088	na	0.023	na	0.175	0.012	0.110	0.122
10. Dog Cr. area (96)	na	2.713	0.623	0.317	1.457	2.587	0.646	1.262	na	–	na	na	na	na	na	na	na	na	na
11. Ney Springs Cr.	na	0.729	0.509	0.944	0.646	0.876	0.396	0.545	na	1.253	–	0.141	na	0.004	na	0.073	0.070	0.003	0.039
12. Soda Creek	na	1.709	1.597	3.082	1.921	0.886	0.854	0.807	na	2.316	0.637	–	na	0.023	na	0.046	0.089	0.011	0.053
13. Castle Creek (96)	na	8.355	5.758	4.409	7.348	8.187	4.995	8.327	na	5.376	5.798	7.494	–	na	na	na	na	na	na
14. Mears Creek (93)	na	11.162	9.297	7.758	11.203	7.754	6.935	7.994	na	7.565	7.466	5.282	9.619	–	na	0.036	0.063	0.002	0.107
15. Hazel Creek (96)	na	3.271	1.391	1.152	2.459	1.471	0.461	1.594	na	1.572	1.070	1.034	4.231	5.273	–	na	na	na	na
16. Slate Creek (93)	na	7.651	4.061	2.345	5.997	5.566	2.850	5.397	na	5.223	4.419	4.930	2.120	5.782	2.216	–	0.141	0.050	0.051
17. Salt Creek (93)	na	8.968	4.002	3.756	5.470	7.312	5.199	5.954	na	5.821	6.977	9.270	11.731	19.111	7.401	7.884	–	0.115	0.149
18. Dog Creek	na	6.602	2.527	0.712	4.856	2.587	0.797	3.359	na	2.340	3.173	3.120	4.906	6.210	1.064	1.638	4.692	–	0.004
19. Mt. Shasta Hatchery	na	25.618	22.560	15.338	20.030	18.071	15.664	19.923	na	16.094	19.192	17.274	20.751	15.814	13.089	12.191	17.259	8.772	–

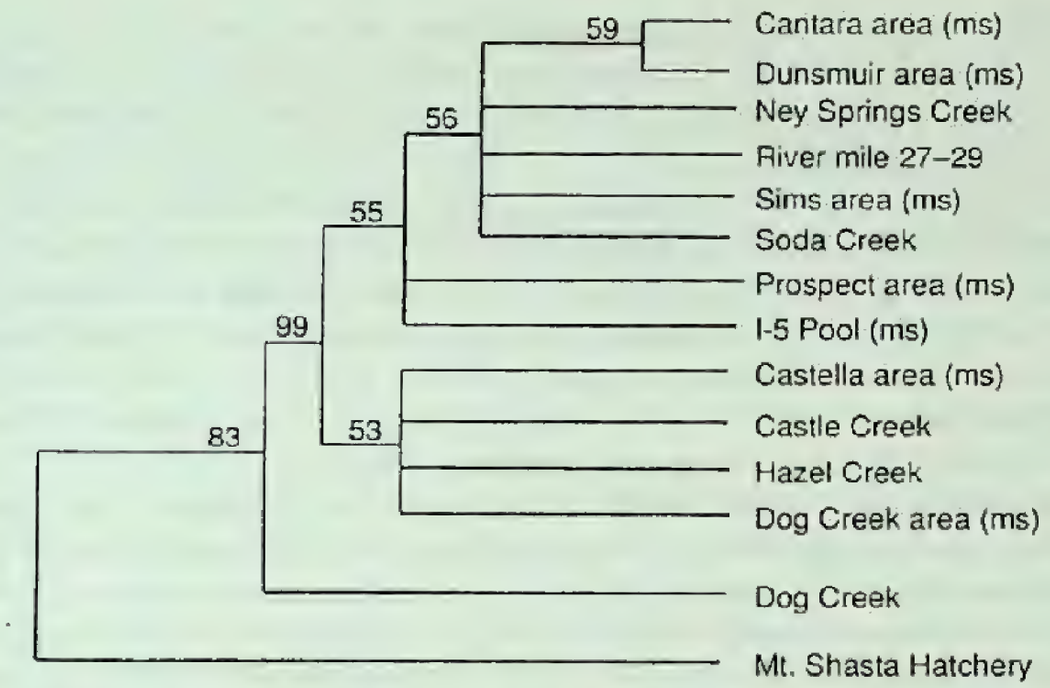

Figure 3. Genetic distance analyses calculated using Goldstein et al. (1995) $\delta\mu^2$ values for the 1996 upper Sacramento River rainbow trout populations (see Table 1). Bootstrap (1,000 replicates) probability values developed from the MICROSAT 1.4 program are given in percentages on the tree branches. ms = mainstem site.

CDFG file photos of rainbow trout moving from the river into the tributaries). In 1993, 2 years after the Cantara spill, our 3-locus analysis of microsatellite differentiation, genetic distance (R_{st}), and mtDNA haplotype frequency distributions showed that most upper mainstem rainbow trout were genetically associated with geographically adjacent tributary trout populations. This suggests colonization of the mainstem directly from upper basin tributaries following the spill.

Five years after the spill, however, we found a very different genetic picture in the upper Sacramento River basin. Genetic analyses using mtDNA sequence and 11 microsatellite loci showed mainstem rainbow trout populations to be well mixed along the total upper river drainage, suggesting significant movement or mixing of rainbow trout in the basin between 1993 and 1996.

Genetic distance models can be used to interpret changes in population structure over long evolutionary time (Nielsen et al. 1994b, FitzSimmons et al. 1995, Jarne and Logda 1996, Nielsen et al. 1997b, Nielsen 1999). They also serve as graphic interpretations of population substructure in closely related or geographically adjacent populations (Angers et al. 1995; Boyce et al. 1996; Nielsen et al. 1997a, 1997b, 1998, 1999; Nielsen 1999; Nielsen and Fountain 1999). The literature contains substantial discussion about the appropriate use and application of genetic distance measures using microsatellites (Goldstein et al. 1995, Takezaki and Nei 1996, Goodman 1997, Ruzzante 1998). In this study, we compared genetic distance results for population subdivision among Sacramento River rainbow trout derived from 2 different distance models.

The expectation of a strict single-step mutation model in $\delta\mu^2$ analyses appears to dampen resolution in closely related groups where the frequency distribution of

alleles is not fixed within sub-basin trout populations. Analyses using R_{st} make no assumptions about mutation rate in relationship to allele size and serve as simple analyses of variance of genetic frequencies among groups when there may be constraints on allele size (Garza et al. 1995, Goodman 1997). This approach appears to fit better in comparisons of geographically proximate populations with recent gene flow or movement between separate population subunits as shown in our 1993 data. Genetic structure among populations of the same species has traditionally been studied using departures of allele frequencies from panmictic expectations (Excoffier et al. 1992). The panmictic genetic structure shown in our 1996 upper Sacramento River rainbow trout samples was supported by analyses of mtDNA and both genetic distance measures using microsatellites.

In our examination of possible hatchery influences on wild trout in the upper Sacramento River basin we provide 2 important findings. In both sampling years (1993 and 1996), we were able to document significant genetic differences between the Mount Shasta Hatchery rainbow trout and wild rainbow trout captured throughout the upper Sacramento River basin, with the exception of fish collected in the lower mainstem reaches. Mount Shasta Hatchery fish were shown to have limited genetic diversity for both mtDNA and microsatellites compared to wild populations of trout in the upper Sacramento River. Our analyses have provided a baseline of genetic information with 11 unique alleles (wild vs hatchery) found at 6 loci. Changes in population genetic structure for Sacramento River wild and hatchery rainbow trout thought to have derived from a common ancestral population in recent times (last 100 years) are probably the result of artificial selection in the husbandry of the cultured fish.

We found only 2 exceptions to the general overall conclusion that hatchery fish have had little influence on wild trout genetics in this basin following the Cantara spill. First, analyses from 1 locus (Ots1) paired 2 upper mainstem trout populations near Dunsmuir (I-5 pool and Financial Avenue collections) in 1996 with the Mount Shasta Hatchery trout. Fisher's comparisons for all 11 loci combined, however, did not support this single-locus association. If any introgression has occurred between hatchery and wild trout over the last 3 years since hatchery fish were reintroduced after the fish-kill event, it appears to have had no significant effect on wild trout genetic diversity at this time. How genetic trends evolve with continued supplementation in this area would be interesting to monitor. Even limited gene flow over long periods of time can influence the genetic diversity in a population (Slatkin 1985)

The 2nd possible hatchery effect found in these analyses involved the frequency of mtDNA haplotypes found in California hatchery rainbow trout strains that were also found in tributary and mainstem habitats in the lower basin above Shasta Lake. These data include a lack of genetic differentiation for mtDNA haplotype frequencies between trout captured at rm 3 and the Mount Shasta Hatchery fish and a lack of microsatellite differences among Dog Creek, the lower mainstem river sampling sites, and the Mount Shasta Hatchery rainbow trout in both 1993 and 1996. These genetic data suggested upstream colonization by hatchery fish from the reservoir into the lower reaches of the upper Sacramento River.

Further support for this hypothesis was provided by trout carrying mtDNA haplotype MYS16 that were found at 2 lower mainstem sites in the 1996 samples. This haplotype has previously only been found in volcano golden trout and Eagle Lake rainbow trout. The MYS16 haplotype is fixed in Eagle Lake rainbow trout samples in our laboratory (Nielsen et al. 1996). Eagle Lake rainbow trout also have a history of supplementation in Shasta Lake, supporting the possibility of upstream movement from the lake into the river by this haplotype. Mitochondrial DNA haplotype distributions suggesting possible introgression by hatchery fish from Shasta Lake into the lower basin appears consistent with several observations of adult hatchery trout in the lower mainstem and Dog Creek over the last 3 years by CDFG (S. Turek, personal communication).

One trout captured in Soda Creek in 1996, however, also carried the MYS16 haplotype, suggesting that it is possible that this haplotype is native to this drainage and represents an ancestral mtDNA lineage that has residualized in several disjunct trout populations in California. Based on mtDNA and microsatellite results from 1993 and 1996, it is also possible that upstream movement of hatchery trout from Shasta Lake into the upper Sacramento River occurred for some time prior to the Cantara spill and continues into the present. Genetic data suggest that the upstream movement of hatchery trout from Shasta Lake has had more of an impact on wild trout genetics in the lower portion of the upper Sacramento River than the supplementation of catchable Mount Shasta Hatchery rainbow trout in the upper mainstem river.

Hatchery stocks emigrating from Shasta Lake appear to be able to reproduce successfully in the lower portion of the river, whereas catchable hatchery trout introduced after the fish-kill into the upper basin show only limited evidence of possible reproductive success. Limited genetic linkage between supplementation hatchery trout and wild trout populations in the upper basin was supported by a lack of genetic differentiation drawn from only 1 microsatellite locus (Ots1). Due to the short period of time (3 years) this study has considered genetic impacts of hatchery introductions in this study area, we recommend continued genetic monitoring in the basin to see if this trend of reproductive isolation between hatchery trout and wild rainbow trout continues in the upper Sacramento River.

In summary, the genetic structure found in 1993 probably resulted from groups of mainstem river trout finding refuge from the toxic spill in local tributaries and mixing with any resident fish found in these habitats. These "invading" populations of mainstem trout probably survived in small groups representing different, closely related demes derived from different population units in the upper Sacramento River before the spill. Genetic isolation of these tributary populations was documented in 1993 with some evidence of the initial recolonization of trout from the tributaries into adjacent mainstem habitats. Genetic evidence for the mixing of these demes by 1996 was interesting and unexpected. The 1996 sample demonstrating panmictic genetic conditions in mainstem and tributary habitats suggests significant movement of fish throughout the river system 5 years after the spill. The time frame for this movement may reflect a recovery of fish food resources within the system.

Competition for open, novel, and productive habitats after recovery of the ecosystem could explain this scattering of fish throughout the river. Whatever the mechanisms, these data suggest that the Cantara metam sodium spill had significant effects on the genetic structure of rainbow trout throughout the entire river system. One interesting question to ask is how long it will take for the trout populations to reach any level of population genetic substructure again within the mainstem river and tributaries following this pattern of recolonization following a fish-kill event.

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POSSIBLE EFFECTS OF THE CANTARA SPILL ON AMPHIBIAN POPULATIONS OF THE UPPER SACRAMENTO RIVER

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Three years after a metam sodium herbicide spill, amphibian species richness on aquatic plots was significantly lower on the Sacramento River than at reference sites on nearby tributaries and the Trinity, Salmon, and Smith rivers. Pacific giant salamanders, *Dicamptodon tenebrosus*, accounted for 88% of all sightings at all locations. The proportion of plots with this species was lower on the Sacramento River than on reference sites at all sampling times (4 months and 1, 2, and 3 years post-spill). Abundance-habitat relationships predicted that Pacific giant salamander density was 0.001 individuals/m^2 before the spill. Three years after the spill, populations were still 93% lower than this estimate. Amphibian species richness on terrestrial plots was not significantly different between the Sacramento River and the reference tributaries at either 4 or 6 months post-spill. Ensatina, *Ensatina eschscholtzii*, accounted for 87% of all sightings. The number of ensatina per plot was greater on the Sacramento River than on the tributaries 6 months post-spill. Ensatina number and species richness on terrestrial plots increased with distance from the spill site at both sample times. Elevation, number of large trees, and area of downed logs accounted for 38% (spring 1992) to 66% (winter 1991) of the variation in ensatina number.

INTRODUCTION

Amphibians are abundant, integral members of terrestrial and aquatic ecosystems (Burton and Likens 1975, Bury et al. 1980), often making up the greatest fraction of the vertebrate biomass (Blaustein and Wake 1990) and having significant trophic effects on invertebrates and other prey (Parker[1] 1992). The susceptibility of

[1] Parker, M.S. 1992. Feeding ecology of larvae of the Pacific giant salamander (*Dicamptodon tenebrosus*) and their role as top predator in a headwater stream benthic community. Ph.D. Dissertation, University of California, Davis, California, USA.

amphibians to environmental degradation has recently been highlighted during investigations into world-wide amphibian declines (Halliday and Heyer 1997) and has prompted land managers to consider amphibians as bio-indicators of ecosystem health.

The release of 72,000 liters of metam sodium on 14 July 1991 into the upper Sacramento River at the Cantara Loop had potentially devastating effects on local amphibian populations. Although the effects of metam sodium on amphibians have not been clinically tested, trials have shown that the chemical causes death in aquatic invertebrates and fish (CDFG[2] 1991). Observations of dead Pacific giant salamanders, *Dicamptodon tenebrosus*, on river banks after the spill (A. Lind, USDA Forest Service, Redwood Sciences Laboratory, personal communication; D. Smith and J. Miller, California Department of Fish and Game [CDFG] personal communication) provide strong evidence that aquatic amphibians are similarly affected by metam sodium or its by-products.

The purpose of this study was to determine how the Cantara spill affected aquatic and terrestrial populations of amphibians in the upper Sacramento River watershed. Because data on amphibian species richness and abundance were not available from the area prior to the spill, we used reference sites to estimate pre-spill species richness and abundance. Our objectives were to 1) estimate pre-spill amphibian richness and abundance by comparing populations on the Sacramento River with those at reference sites on nearby tributaries and on other rivers in northern California, 2) determine the suitability of reference sites by comparing habitat on the Sacramento River with habitat at reference locations, and 3) monitor a subset of the species to determine rates of population recovery following spill impacts.

METHODS

Station and Plot Placement

Sacramento River

We used cluster sampling to estimate amphibian species richness and abundance. Beginning at the spill site and moving downstream, we systematically established stations on the river bank in blocks of suitable habitat (i.e., areas without human disturbances and with a shallow enough slope to survey safely). In 1991, we established 5 stations between the spill site and Sims (Fig. 1). In 1993, we added 2 stations downstream near Gibson and La Moine. Within each station, we systematically placed 5 terrestrial and 5 aquatic plots. Systematic placement of stations and plots is more efficient to execute in the field than simple random sampling, reduces selection errors, and provides greater information per unit cost (Scheaffer et al. 1986). In this study, it also ensured that the distance between plots was maximized within

[2]CDFG. 1991. Natural Resource Damage Assessment Plan, Sacramento River: Cantara Spill, Shasta and Siskiyou counties. California Department of Fish and Game, Redding, California, USA.

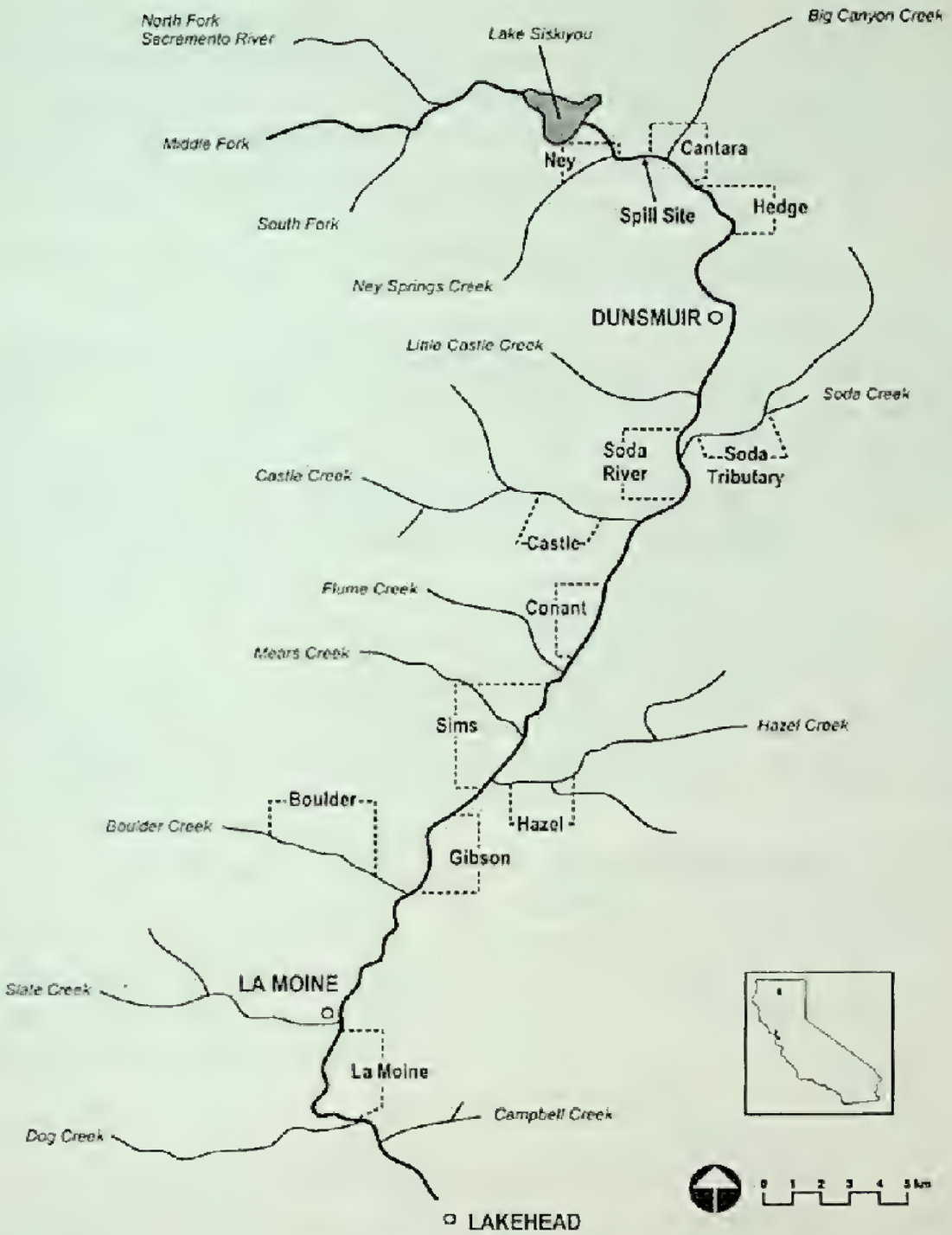

Figure 1. Locations of survey stations on the Sacramento River and reference tributaries. Gibson and La Moine stations were surveyed in 1993 and 1994 only.

stations to reduce the chance of animals moving among plots. Terrestrial plots, 140 x 40 m, were established at 300-m intervals within each station. Aquatic plots, 140 x 10 m, were established immediately adjacent and parallel to the terrestrial plots.

Reference Sites

Reference stations were placed in suitable habitat upstream of the spill site and along tributaries. Only 1 reference station at the mouth of Ney Springs Creek (Fig. 1) could be established upstream of the spill because Lake Siskiyou Dam prevented us

from continuing upriver. Significant levels of metam sodium, presumably transported by up-canyon winds, were later measured in leaf-litter samples from this area (CDFG[2] 1991; J. Nelson, CDFG, personal communication). For this reason, we considered all terrestrial survey plots at the Ney station to be in the impact area. Because no detectable levels of the herbicide were found in the water upstream of the spill site, we considered the aquatic survey plots in the station to be in a reference area. On the tributaries, we randomly selected 4 stations that had similar vegetation and aquatic habitat characteristics to the river station sites. Methods for placing plots within reference station boundaries were the same as for the river stations.

In 1993, we increased aquatic survey sampling effort by adding 4 additional plots to each station. The new plots were added in suitable habitat between the existing 5 plots and the minimum distance between plots was reduced to 80 m. These additions brought the total number of aquatic plots to 63 (7 stations with 9 plots each) on the Sacramento River and 45 (5 stations with 9 plots each) on the tributaries.

Also in 1993, we added aquatic reference stations on 3 other rivers in northern California: 2 stations on the Trinity River above Clair Engle Lake, 2 stations at the confluence of the North Fork and main stem of the Salmon River near Forks of the Salmon, and 2 stations on the Smith River near Gasquet (Fig. 2). These rivers were chosen based upon similarity in hydrology, temperature, and levels of human disturbance to the Sacramento River. All stations were located in habitat with 1) abundant embedded cobble, 2) moderate flow rate, and 3) a high proportion of

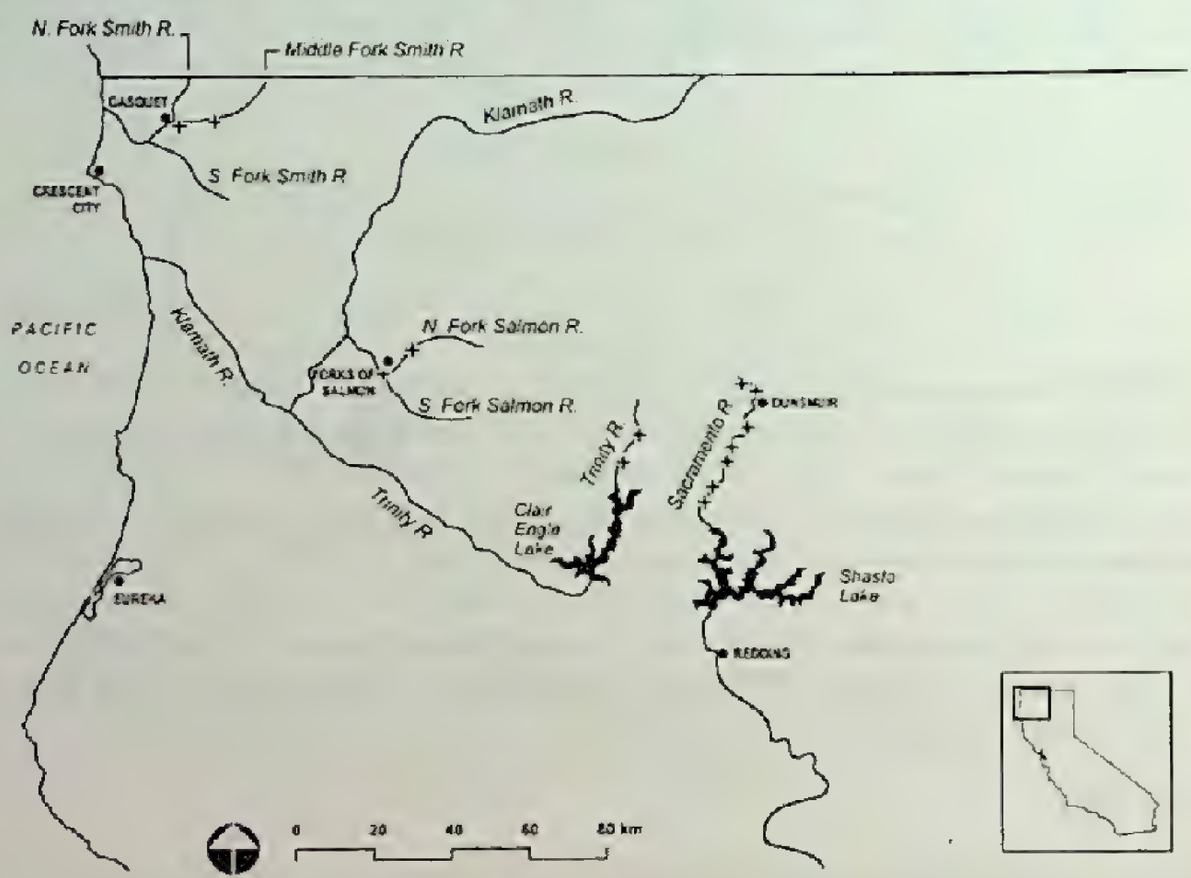

Figure 2. Locations of survey stations (+) on the Sacramento River and reference rivers.

shallow water (<61 cm) within 10 m of the bank. A cobble-dominated substrate is typical of the Sacramento River and is a habitat condition closely associated with Pacific giant salamanders (Welsh[3] 1993). Moderate flow rate and shallow water are necessary to allow surveyors to safely search for amphibians. Methods for placing stations within suitable habitat and establishing 9 aquatic plots within each station were the same as those for the Sacramento River.

Aquatic and Terrestrial Amphibian Surveys

From 7 to 11 December 1991 and 8 to 11 July 1992, we surveyed aquatic plots for amphibians on 5 Sacramento River and 5 tributary stations. From 16 to 26 August and 2 to 4 September 1993, we surveyed 7 Sacramento River stations and 6 reference river stations. From 2 to 24 August and 6 July to 1 August 1994, we surveyed the 7 Sacramento River and 5 tributary stations. On each plot, 1–4 surveyors searched for amphibians under submerged rocks and logs using dipnets, potato rakes, and viewing boxes.

All 50 terrestrial plots were surveyed by 8 biologists (2 teams of 4 persons) between 16 November and 11 December 1991 and again from 17 to 25 March 1992. Each plot was subdivided into four 140 x 10-m subsections paralleling the waterway. Each biologist searched 1 subsection for 1 hour by turning over all cover objects, including logs, rocks, leaf litter, and bark on stumps and logs. Surveyors used 25-cm potato rakes to rake through leaf litter and turn rocks and logs. Cover objects were replaced in the their original positions.

On both aquatic and terrestrial plots, captured amphibians were identified, weighed, measured, sexed, aged, and released. If an amphibian was captured, surveyors recorded the species and estimated snout–vent length (SVL). Time spent capturing and handling amphibians was not included in total search time. To minimize measurement bias among surveyors, surveyors were frequently rotated among plots and reassigned to different teams.

Habitat Surveys

We measured habitat variables to determine whether tributary plots were suitable reference sites for the Sacramento River plots. Because habitat requirements vary among species, we focused on habitat variables known to be correlated with the 2 most abundant species captured during plot surveys: Pacific giant salamanders on aquatic plots and ensatina, *Ensatina eschscholtzii*, on terrestrial plots. For Pacific giant salamanders, we chose 10 aquatic habitat variables previously reported to be correlated with abundance: aspect, slope, stream width, proportion undercut bank, proportion cobble substrate, percent canopy cover, vegetation overhang, bank rating, proportion riffle habitat, and embeddedness (Hawkins et al. 1983, Parker 1991, Welsh[3]

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[3] Welsh, H.H., Jr. 1993. A hierarchical analysis of the ecological relationships of four amphibians from forested habitat of northwestern California. Ph.D. Dissertation, University of California, Berkeley, California, USA.

1993). We chose 7 habitat variables previously reported to be correlated with ensatina abundance: aspect, slope, elevation, plant cover class, rock cover class, number of hardwood trees >50 cm in diameter at breast height (DBH), and number of trees >80 cm DBH (Aubry and Hall[4] 1991, Bury et al.[5] 1991, Corn and Bury[6] 1991, Gilbert and Allwine[7] 1991, Welsh and Lind[8] 1991).

Aquatic Habitat Survey

Between 20 August and 9 October 1992, we conducted aquatic habitat surveys at all stations. Slope (0– 90°) and aspect (0 [N]–359°) were measured lengthwise over at least half of the plot using a clinometer and compass, respectively. Aspect measurements were transformed by subtracting 135° and sine-transforming the resulting number (Zar 1984) to provide a polar ordination of aspect along a moisture gradient from northeast (45°, transformed aspect = -1) to southwest (225°, transformed aspect = 1). All other aquatic habitat parameters were estimated from transects oriented perpendicular to the bank and placed at 5-m intervals (vegetation overhang, stream/plot width, proportion undercut bank, bank rating) or 10-m intervals (canopy cover, embeddedness, proportion cobble substrate).

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[4] Aubry, K.B. and P.A. Hall. 1991. Terrestrial amphibian communities in the southern Washington Cascade range. Pages 327-338 *in*: L.F. Ruggiero, K.G. Aubry, A.B. Carey, and M.H. Huff, technical coordinators. Wildlife and vegetation of unmanaged Douglas-fir forests. General Technical Report PNW-GTR-285, USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon, USA.

[5] Bury, R.B., P.S. Corn, K.B. Aubry, F.F. Gilbert, and L.L.C. Jones. 1991. Aquatic amphibian communities in Oregon and Washington. Pages 353-363 *in*: L.F. Ruggiero, K.G. Aubry, A.B. Carey, and M.H. Huff, technical coordinators. Wildlife and vegetation of unmanaged Douglas-fir forests. General Technical Report PNW-GTR-285, USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon, USA.

[6] Corn, P.S. and R.B. Bury. 1991. Terrestrial amphibian communities in the Oregon coast range. Pages 305-317 *in*: L.F. Ruggiero, K.G. Aubry, A.B. Carey, and M.H. Huff, technical coordinators. Wildlife and vegetation of unmanaged Douglas-fir forests. General Technical Report PNW-GTR-285, USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon, USA.

[7] Gilbert, R.G. and R. Allwine. 1991. Terrestrial amphibian communities in the Oregon Cascade range. Pages 319-324 *in*: L.F. Ruggiero, K.G. Aubry, A.B. Carey, and M.H. Huff, technical coordinators. Wildlife and vegetation of unmanaged Douglas-fir forests. General Technical Report PNW-GTR-285, USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon, USA.

[8] Welsh, H., Jr. and A.J. Lind 1991. The structure of the herpetofaunal assemblage in the Douglas-fir/hardwood forests of northwestern California and southwestern Oregon. Pages 395-413 *in*: L.F. Ruggiero, K.G. Aubry, A.B. Carey, and M.H. Huff, technical coordinators. Wildlife and vegetation of unmanaged Douglas-fir forests. General Technical Report PNW-GTR-285, USDA Forest Service, Pacific Northwest Research Station, Portland, Oregon, USA.

All habitat measurements followed Platts and McHenry[9] (1988), except that stream width was measured as width of the stream minus the width of habitat that could not be surveyed due to depth or swift currents. Maximum stream width was set at 10 m. Canopy cover was measured by averaging 4 densiometer readings facing N, S, E, and W on the bank edge. To estimate substrate and embeddedness, we systematically spaced 0.2 x 0.3-m sampling quadrats along a line oriented perpendicular to stream flow. Within each quadrat, we recorded dominant substrate type (sand/gravel, cobble, boulder, or bedrock) and ranked its embeddedness.

Terrestrial Habitat Surveys

Between 5 and 27 August 1992, we conducted terrestrial habitat surveys on all stations. On each plot, we counted the number of hardwood trees >50 cm DBH and all trees >80 cm DBH. Mean elevation for each plot was recorded using 7.5-minute U.S. Geological Survey topographic maps.

Along 4 transects, each separated by 47 m and oriented perpendicular to the waterway, we measured slope and aspect using a clinometer and compass, respectively. Aspect measurements were transformed as described for the aquatic habitat survey.

Along four 28-m-wide strip transects oriented parallel to the waterway, we systematically placed eighteen 5-m radius circular and 1 x 1-m square quadrats. In the circular quadrats, we recorded width and length (cm) of all downed woody debris >10 cm in diameter, and rock cover class for rocks >10 cm in diameter (class 1 = 0 rocks, 2 = 1–5 rocks, 3 = 6–10 rocks, 4 = 11–25 rocks, and 5 = >25 rocks). In the square quadrats, we estimated percent cover for plants <0.5 m tall using the following cover classes: 1= 0–5% cover, 2 = 5–25% cover, 3 = 25–50% cover, 4 = 50–75% cover, 5 = 75–100% cover (Braun-Blanquet 1932).

Data Analysis

Data from the terrestrial and aquatic surveys were analyzed separately. We measured species richness as the number of amphibian species found in a plot. For the most common species, we also made abundance comparisons. Ensatina occurred in large numbers on many terrestrial plots, so abundance was estimated as the number of individuals per plot. Pacific giant salamanders occurred in low numbers on aquatic plots, so abundance was indexed as the frequency of plots with individuals present. Comparisons among frequencies were made using chi-square tests (Zar 1984). Species richness, abundance, and habitat variables were compared using the multi-stage sample means and 95% confidence intervals (Cochran 1977, Scheaffer et al. 1986). Relationships between ensatina number and habitat variables in winter 1991 and spring 1992 were evaluated using stepwise regression. For all regression analyses, the

[9] Platts, W.S. and M.L. McHenry. 1988. Density and biomass of trout and char in western streams. General Technical Report INT-241. USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah, USA.

dependent variable was examined for normality and tested for homoscedasticity against the independent variables. Variables were log or arcsine transformed as appropriate (Zar 1984).

To analyze differences in the age structure of Pacific giant salamanders among survey locations, we divided salamanders into 4 age classes using SVL: 1) 1st-year larvae = 0–65 mm, 2) 2nd-year larvae = 66–85 mm, 3) 3rd-year larvae = 86–115 mm, and 4) paedomorphic adults = 115 mm. These age estimates were based on summer surveys in other areas of the salamander's range (Nussbaum and Clothier 1973, Parker 1991). Low numbers of individuals captured on the Sacramento River did not allow meaningful statistical comparisons of age distribution between the impacted and reference locations. A chi-square test was used to compare size-class distributions between the reference rivers and the tributaries. Paedomorphic adults were excluded from the analysis because the small expected frequencies for this size class would have resulted in a biased chi-square value (Zar 1984).

We estimated pre-spill numbers of Pacific giant salamanders on the Sacramento River by using stepwise regression analysis to develop an equation that predicted salamander abundance based on reference site habitat variables. The number of Pacific giant salamanders found on each tributary plot was standardized by the proportion of surveyable area on the plot and the proportion of 1 hour that the plot was surveyed. Because many plots did not contain Pacific giant salamanders, many abundance-habitat variable relationships were heteroscedastic and could not be rectified with log transformations. Therefore, for the stepwise regression, we used only plots where Pacific giant salamanders were found. We used only the summer 1994 data, because the number of plots with salamanders in 1992 was too few for our regression analysis.

After developing the final "best" equation using the stepwise regression analysis, we inserted habitat values from each Sacramento River plot. Summing across all plots resulted in an estimated number of salamanders prior to the spill. We then adjusted our estimate for the "no occurrence" plots by multiplying salamander number by the average proportion of reference river plots with salamanders. Dividing by the number of plots gave an average number of salamanders per plot; 95% confidence intervals were approximated by substituting the slope of the equation with ± 2 SE of the slope.

RESULTS

Aquatic Surveys

Two of the 10 aquatic habitat variables differed between the Sacramento River and tributaries. Tributary plots were narrower and steeper than Sacramento River plots based on a comparison of multi-stage sampling means and 95% confidence intervals (Fig. 3). Tributary plots also tended to have less overhanging vegetation, although this difference was not significant. Confidence intervals varied between 2% (river width) and 65% (tributary vegetation overhang) of the means.

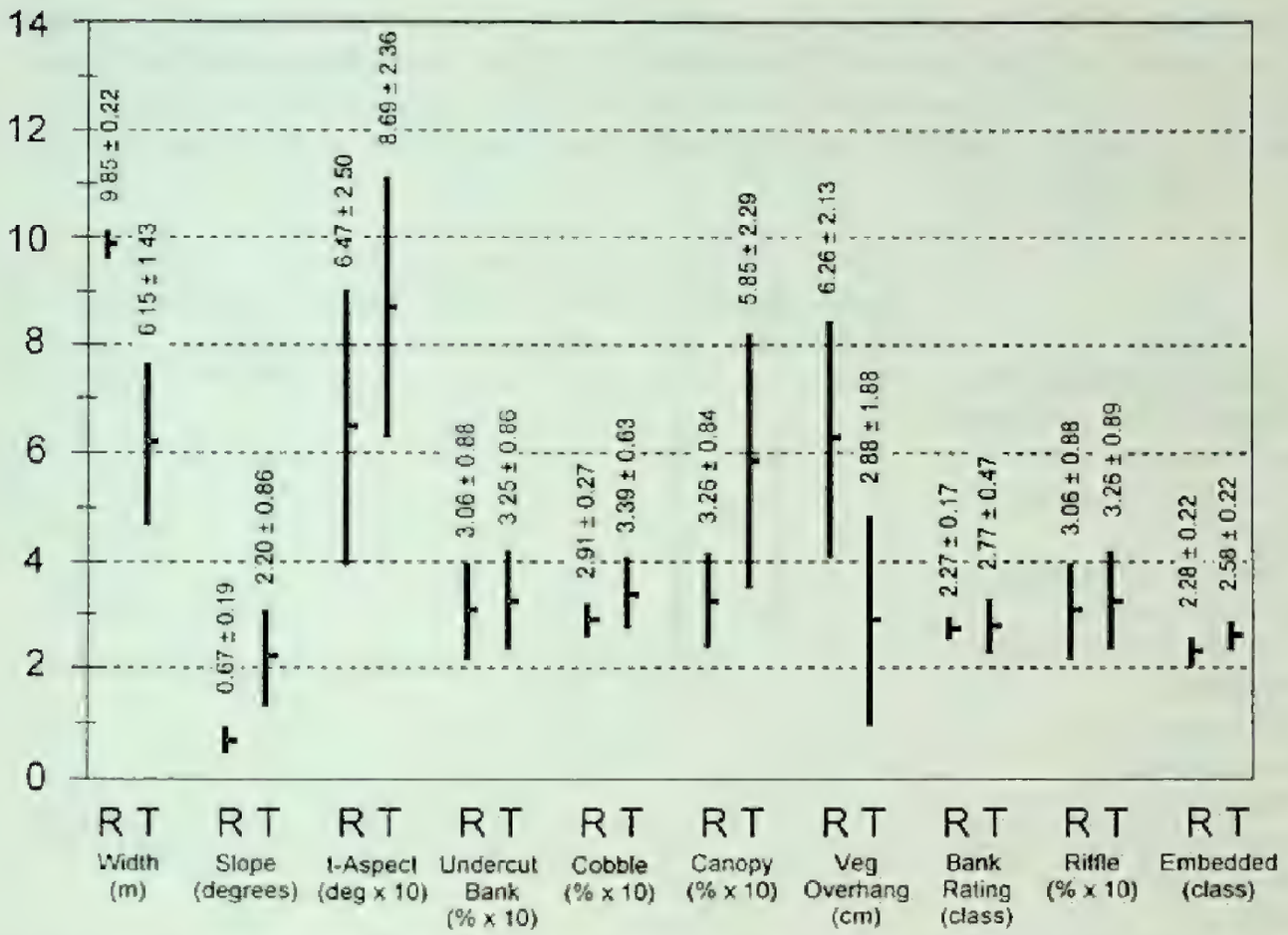

Figure 3. Comparison of aquatic habitat variables known to be correlated with Pacific giant salamander abundance on the Sacramento River (R) and reference tributaries (T). Multi-stage sample means and 95% confidence intervals are shown. Units of the y-axis are indicated under each variable name on the x-axis.

Amphibian Richness at Aquatic Stations

During aquatic surveys, we identified 8 amphibian species (Table 1). Six species were found on the Sacramento River and on the tributaries, and 4 species were found on the reference rivers. Pacific giant salamanders were found at all survey locations and represented 88% of total captures. Two dead Pacific giant salamanders found on a Soda Creek tributary plot and on a Smith River plot were not used in subsequent calculations of species richness or abundance.

Mean species richness on the Trinity and Smith rivers in 1993 was greater than during any survey period on the Sacramento River (Fig. 4) based on comparisons of multi-stage sample means and 95% confidence intervals. Mean species richness was greater on all reference rivers combined than on the Sacramento River in 1993. In 1992, mean species richness on the tributaries was not greater than that on the Sacramento River, although the variances of both estimates were too great to detect even moderate differences between the means. Increased sampling effort in 1994 decreased the variances and mean species richness was greater on the tributaries than on the Sacramento River (Fig. 4). Although average species richness showed an apparent decline on the Sacramento River between 1992 and 1994, the trend was not significant (Fig 4).

Table 1. Amphibians occurring in the upper Sacramento River watershed. Species observed during aquatic (A) and terrestrial (T) surveys in the impact area (Sacramento River) and reference sites (Sacramento River tributaries and the Trinity, Salmon and Smith rivers) are indicated with an "x." Tadpoles, except for tailed frogs, were not tallied. A "+" indicates that only tadpoles were found.

	Sacramento River		Sacramento tributaries		Trinity River	Salmon River	Smith River	Total Number (%)	
Species	A	T	A	T	A	A	A	A	T
Pacific giant salamander, *Dicamptodon tenebrosus*	x	x	x		x	x	x	284(88.3)	1(0.2)
Rough-skinned newt, *Taricha granulosa*		x	x	x				14(4.2)	18(3.2)
Ensatina, *Ensatina eschscholtzii*		x		x					497(87.3)
Black salamander, *Aneides flavipunctatus*		x	x	x				1(0.3)	28(4.9)
Tailed frog, *Ascaphus truei*	x		x				x	3(0.9)	
Western toad, *Bufo boreas*	x		x			x		1(0.3)	
Pacific treefrog, *Hyla regilla*	+	x		x			+		11(1.9)
Bullfrog, *Rana catesbeiana*	x							1(0.3)	
Foothill yellow-legged frog, *Rana boylii*	x		x	x	x	x	x	18(5.7)	14(2.5)

Amphibian Abundance on Aquatic Stations

In 1993, Pacific giant salamanders were found more frequently on plots on all reference rivers combined than on the Sacramento River (χ^2 = 41.81, df = 1, P <0.0001) (Fig. 5). The frequency of plots with Pacific giant salamanders on the Salmon River in 1993 was greater than the frequency on the Sacramento River in 1994 (χ^2 = 7.04, df = 1, P = 0.008). Frequencies of occurrence on the Sacramento tributary stations were greater than those found on the Sacramento River in 1992 (χ^2 = 6.13, df = 1, P = 0.013) and 1994 (χ^2 = 36.66, df = 1, P <0.0001).

During 1992 summer surveys, only 1st-year larvae (20–65 mm SVL) were found on the Sacramento River (Fig. 6a). In 1993, 2nd- and 3rd-year larvae also were found on the Sacramento River. The frequencies of the 3 age classes found during the 1993 surveys on the reference rivers were not significantly different (χ^2 = 0.30, df = 2, P = 0.86) from those on the tributary plots in 1994 (Fig. 6b and c).

Recovery Estimates for Pacific Giant Salamander

The only aquatic variable that was significantly related to the number of Pacific giant salamanders was the proportion of undercut bank (F = 5.17; df = 1, 13; P = 0.041).

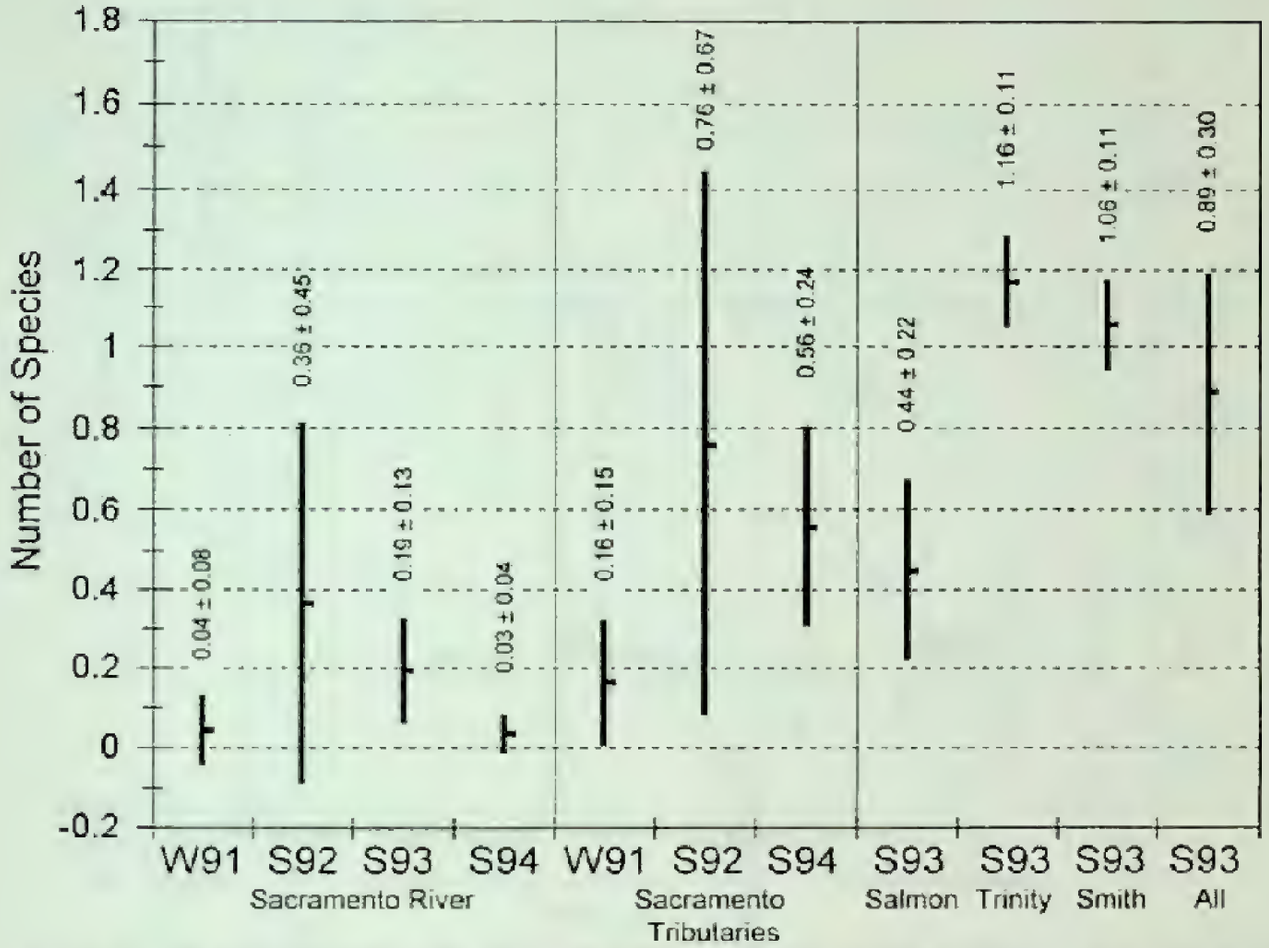

Figure 4. Species richness on aquatic plots on the Sacramento River and reference sites. Multi-stage means and 95% confidence intervals are shown. W or S indicates whether the survey was conducted during the winter or summer. "All" indicates the mean and confidence interval for the Salmon, Trinity, and Smith rivers combined.

This variable accounted for 23% of the variation in salamander number. Using the regression equation log(standardized Pacific giant salamander number) = 0.405 + 2.427(proportion of undercut bank) and the average proportion of plots containing salamanders on the reference rivers (64%) (Fig. 5), we estimated that an average of 1.4 salamanders/plot (0.001 salamanders/m^2) may have lived in the Sacramento River before the spill. Over the 37 months of surveys, the trend in number of salamanders per plot was not significant ($F = 0.19$; df = 1, 2; $P = 0.703$) (Fig. 7).

Terrestrial Surveys

None of the terrestrial habitat variable means were different between the Sacramento River and tributary stations based on a comparison of multi-stage sample means and 95% confidence intervals (Fig. 8). The 95% confidence intervals varied between 8% (tributary plant cover class) and 83% (river woody debris) of the habitat means.

Amphibian Richness on Terrestrial Stations

During winter 1991 and spring 1992 terrestrial surveys, we found 6 amphibian species on Sacramento River and tributary stations (Table 1). Each habitat supported

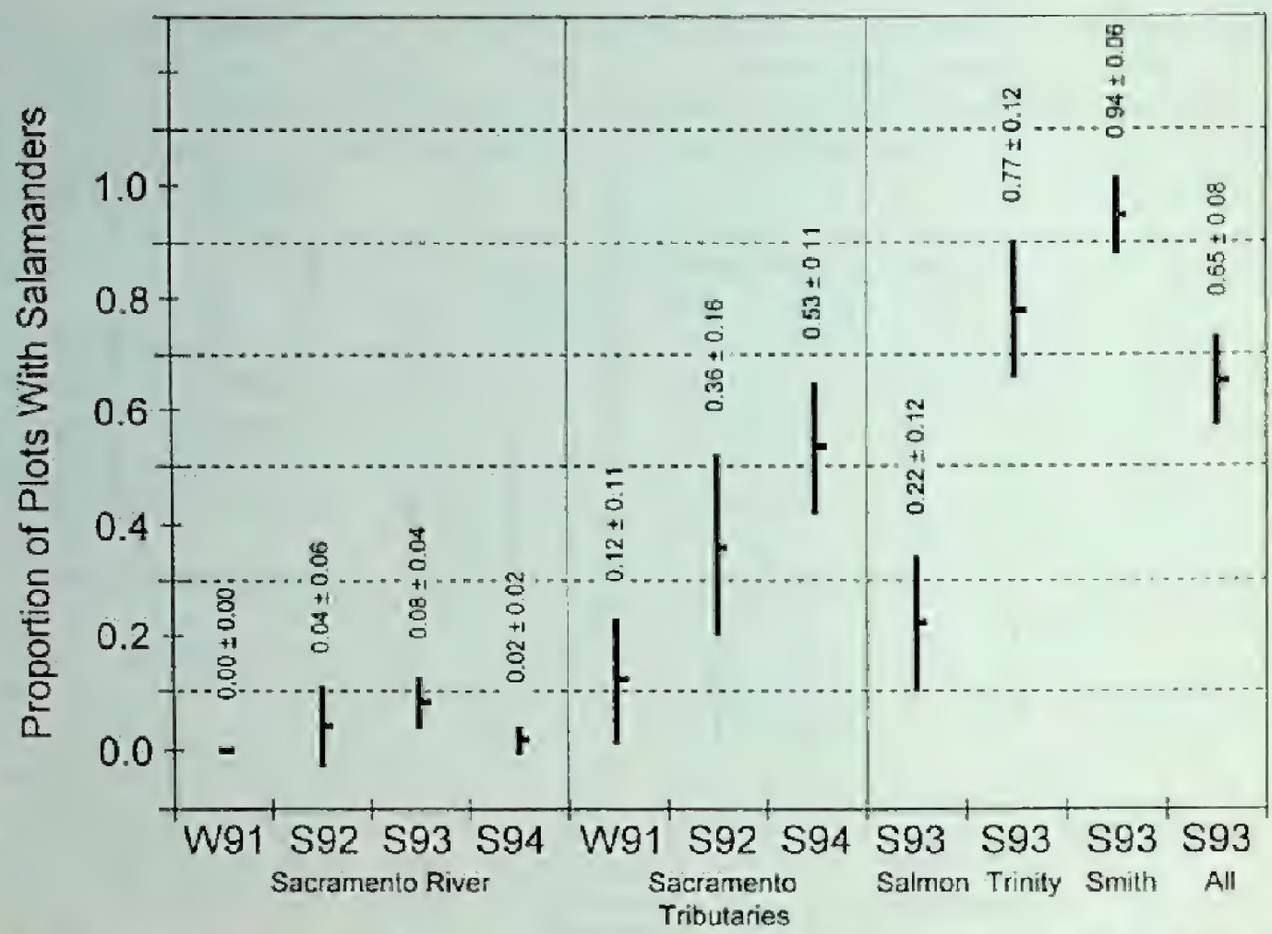

Figure 5. Proportion of plots with Pacific giant salamanders on the Sacramento River and reference sites. Proportion and 95% confidence intervals are shown. W or S indicates whether the survey was conducted during the winter or summer. "All" indicates the mean and confidence interval for the Salmon, Trinity, and Smith rivers combined.

5 species. Ensatina accounted for 87% of all amphibians observed during terrestrial surveys. Black salamanders, *Aneides flavipunctatus*, were the 2nd most abundant amphibian, accounting for 5% of all captures.

Mean species richness was not different between the 2 areas during winter 1991 or spring 1992 surveys. Species richness, however, increased with distance from the spill site in both winter 1991 and spring 1992 surveys (Fig. 9).

Amphibian Abundance on Terrestrial Stations

The number of ensatina per plot was greater on Sacramento River stations than on tributary stations during the spring 1992 surveys, but was not different during the winter 1991 surveys. The number of ensatina per plot increased with distance downriver from the spill site during both surveys (Fig. 10).

Stepwise regression of terrestrial habitat variables on ensatina number indicated that ensatina number was significantly and negatively related to elevation and significantly and positively related to the number of trees >80 cm DBH (winter 1991 and spring 1992 surveys) and with the amount of downed wood >10 cm diameter (winter 1991 survey) (Table 2). Both elevation and number of large trees were significantly related to distance from the spill site (elevation: $F = 1123.37$; $df = 1, 28$;

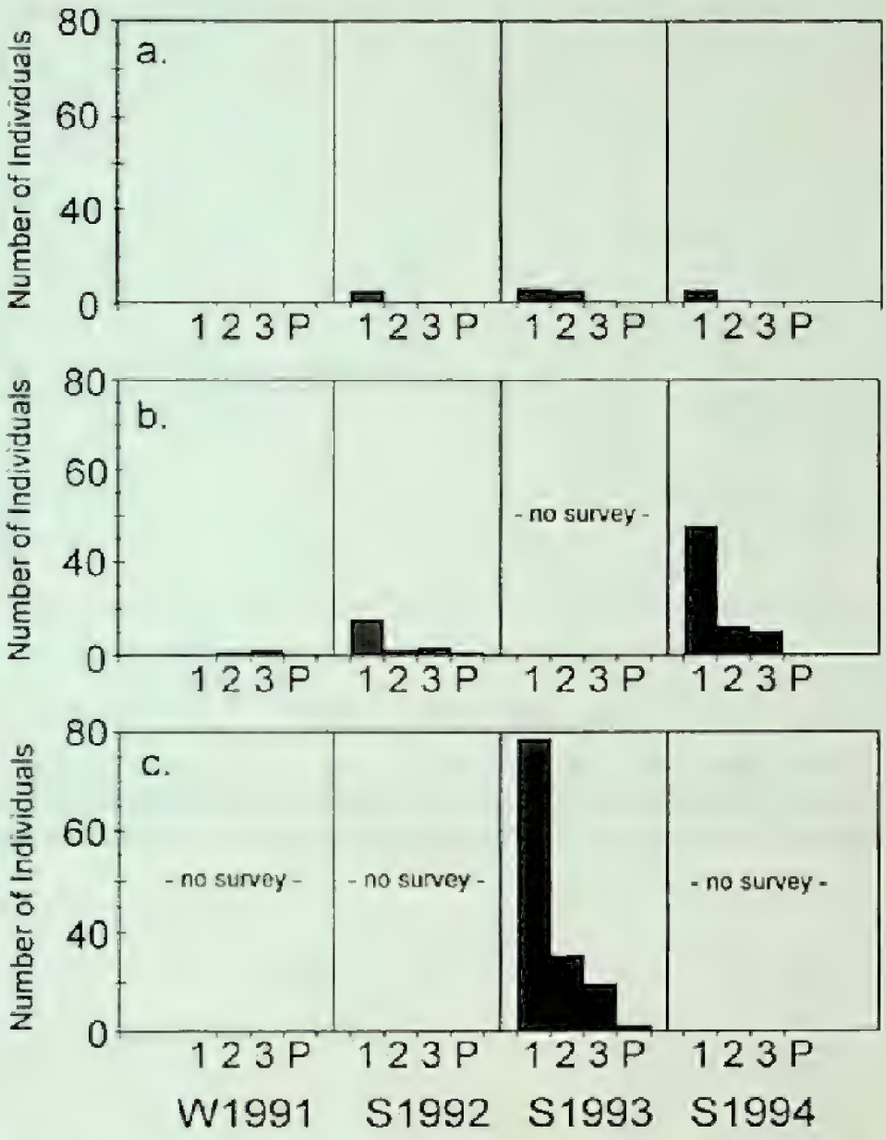

Figure 6. Number of 1st, 2nd, 3rd, and 4th-year (P) Pacific giant salamanders observed during aquatic surveys on the (a) Sacramento River, (b) reference sites on the Sacramento River tributaries, and (c) reference sites on the Salmon, Trinity, and Smith rivers. W or S indicates whether the survey was conducted during the winter or summer.

$P < 0.0001$; number of large trees: F = 3.8378; df = 1, 28; $P = 0.060$). In winter 1991, 67% of the variation in ensatina number could be attributed to these variables. In spring 1992, 38% of the variation was accounted for by variation in elevation and number of large trees (Table 2). Forward, back, or mixed stepping procedures for the stepwise regression analysis yielded the same best fit model.

DISCUSSION

Estimating impacts to populations depends on our ability to find and identify species. Using a combination of aquatic and terrestrial surveys, we found 9 of the 12 species expected to occur in the region. The undetected species, long-toed salamander, *Ambystoma macrodactylum*; red-legged frog, *Rana aurora*; and cascade frog, *R. cascadae*, are usually found in ponds, small streams, and other places with quiet water, which did not commonly occur in our survey stations. Only 2 species, ensatina

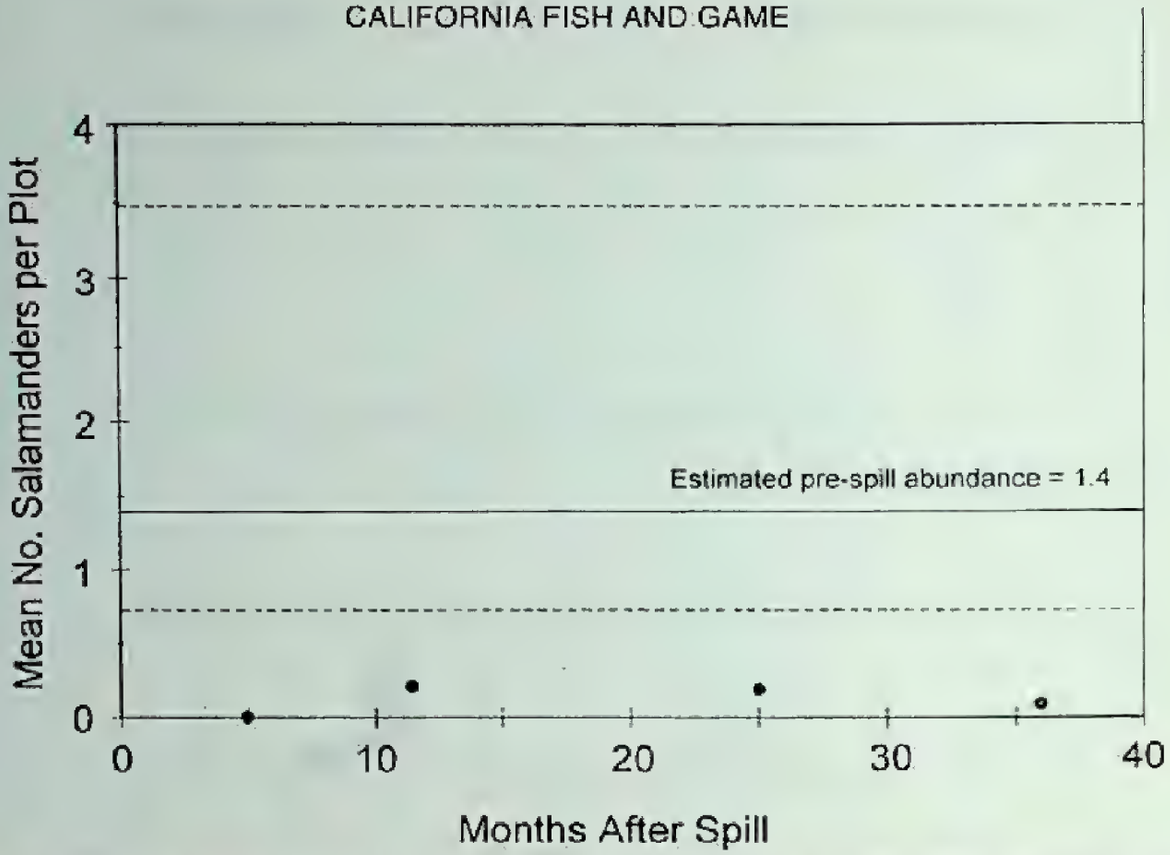

Figure 7. Mean number of Pacific giant salamanders per plot up to 37 months after the Cantara Spill on the upper Sacramento River and the predicted number of pre-spill Pacific giant salamanders per plot (solid line). Dotted lines mark the 95% confidence interval for the predicted value.

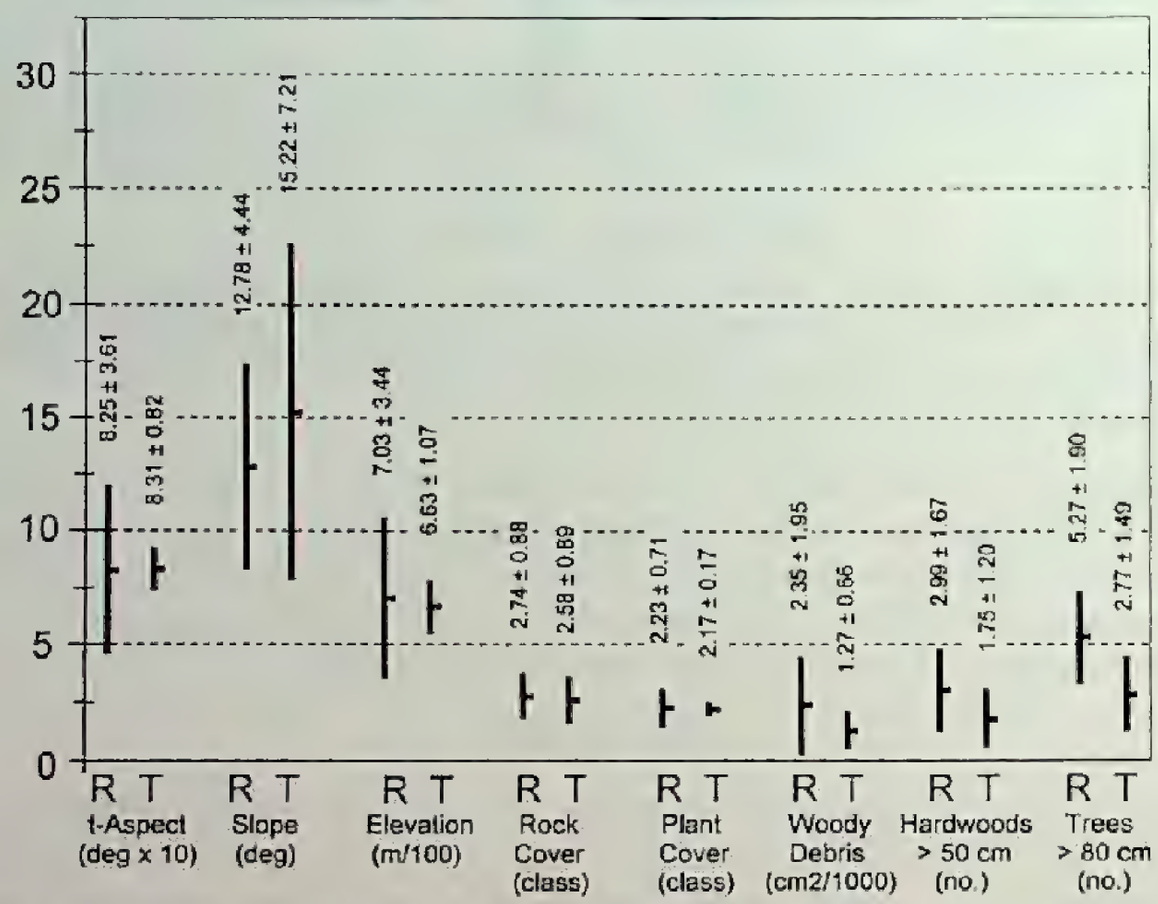

Figure 8. Comparison of terrestrial habitat variables known to be correlated with ensatina abundance on the Sacramento River (R) and reference tributaries (T). Multi-stage sample means and 95% confidence intervals are shown. Units of the y-axis are indicated under each variable name on the x-axis.

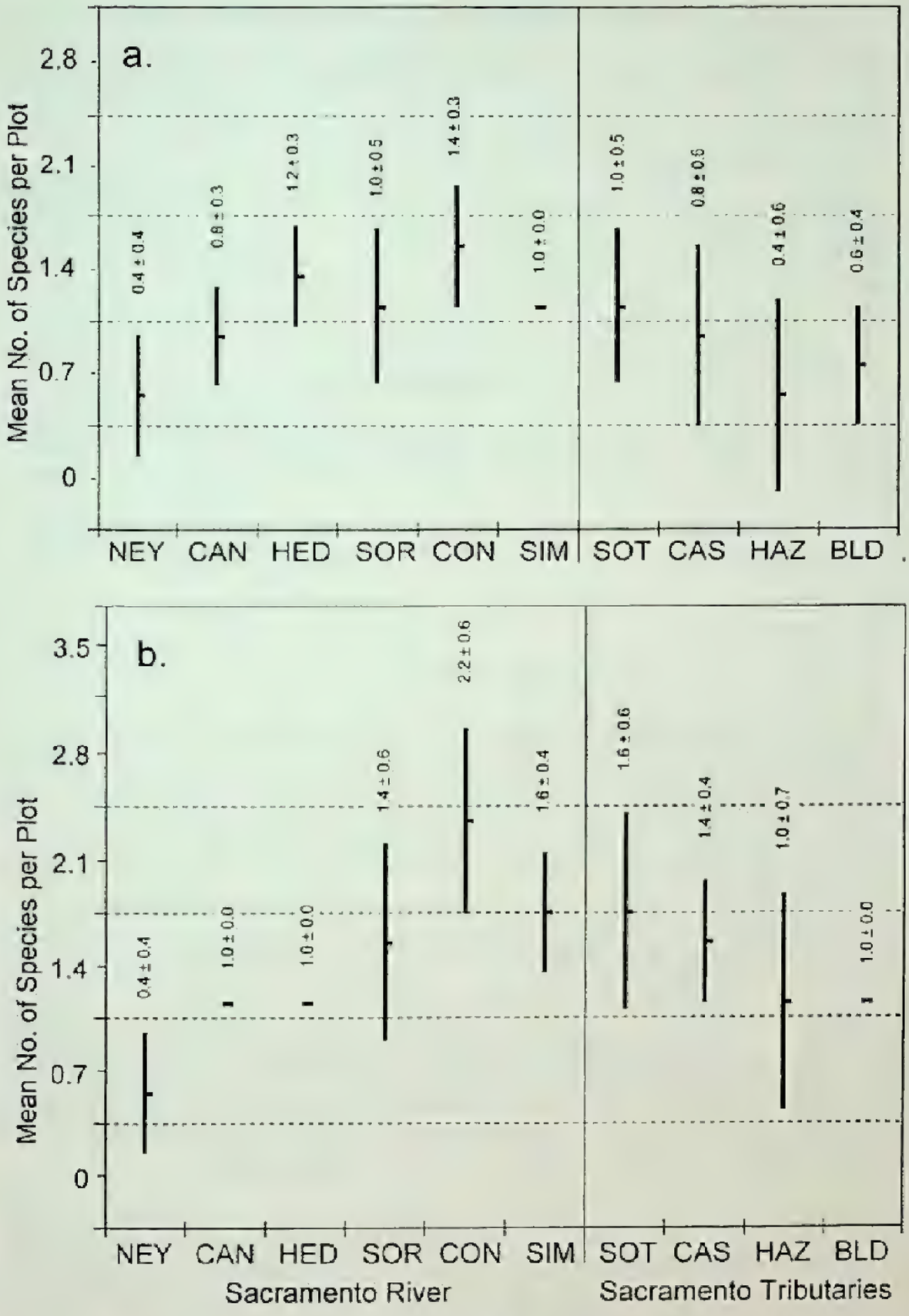

Figure 9. Amphibian species richness on terrestrial plots at each station on the Sacramento River (Ney = NEY, Cantara = CAN, Hedge = HED, Soda River = SOR, Conant = CON, and Sims = SIM) and reference sites on nearby tributaries (Soda Creek = SOT, Castle Creek = CAS, Hazel Creek = HAZ, and Boulder Creek = BLD) during winter 1991 (a) and spring 1992 (b). Multi-stage sample means and 95% confidence intervals are shown. The vertical line separates river and tributary stations which are ordered left to right by increasing distance from the spill site.

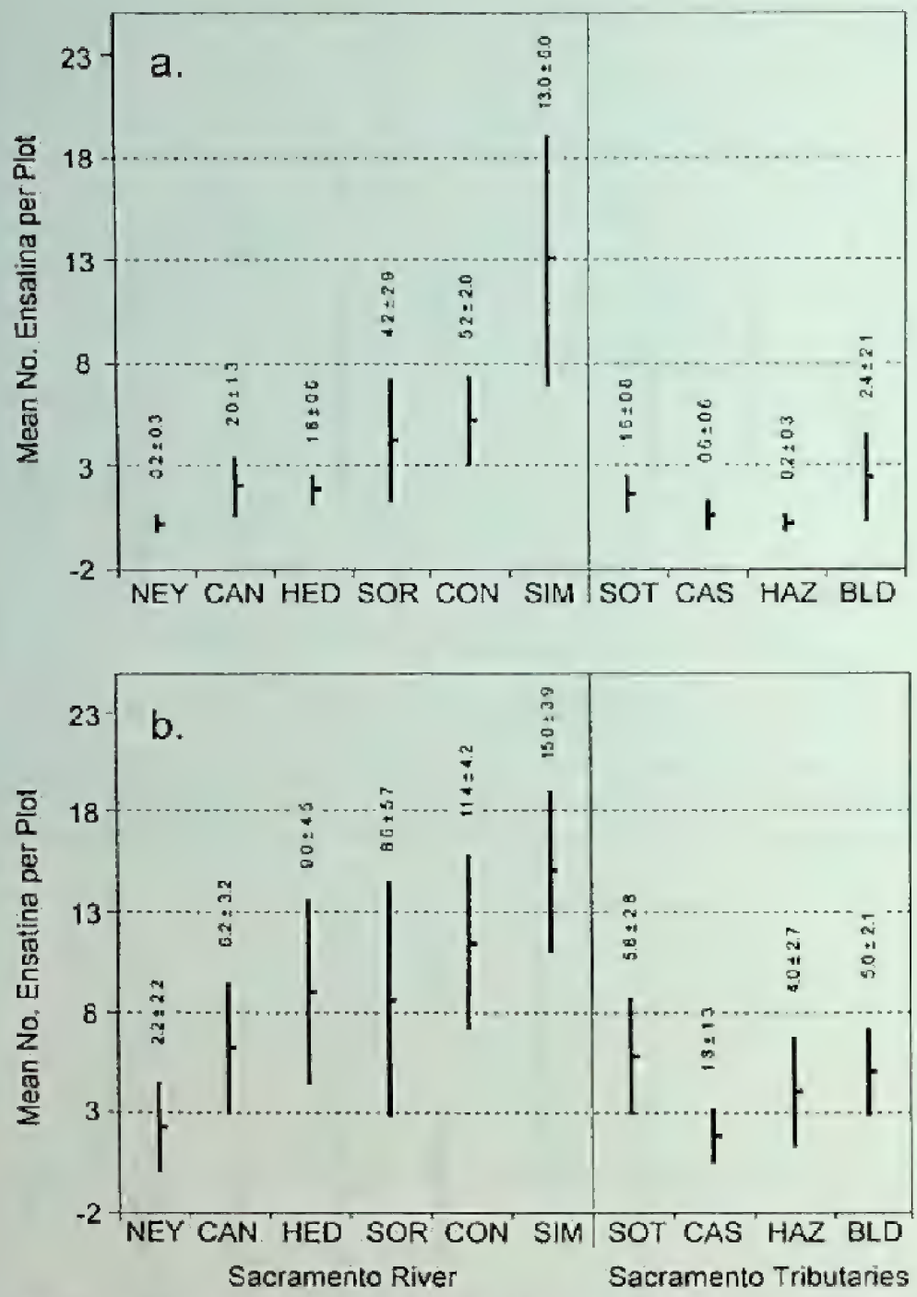

Figure 10. Number of ensatina on terrestrial plots at each station on the Sacramento River (Ney = NEY, Cantara = CAN, Hedge = HED, Soda River = SOR, Conant = CON, and Sims = SIM) and reference sites on nearby tributaries (Soda Creek = SOT, Castle Creek = CAS, Hazel Creek = HAZ, and Boulder Creek = BLD) during winter 1991 (a) and spring 1992 (b). Means and 95% confidence intervals are shown. The vertical line separates river and tributary stations which are ordered left to right by increasing distance downriver from the spill site.

and Pacific giant salamander, were found in sufficient numbers to evaluate possible impacts. Even for these species, ability to detect animals that are present can be difficult. Pilot studies designed to determine the detectability of Pacific giant salamanders on survey plots showed that only 30–45% of the animals are found using the standard survey procedures used in this study (Luke and Sterner[10] 1995).

[10] Luke, C. and D. Sterner. 1995. Cantara Bridge chemical spill: 1994 aquatic amphibian survey. Unpublished report prepared for California Department of Fish and Game by BioSystems Analysis, Inc., Santa Cruz, California, USA.

Table 2. Results of multiple regression analyses for number of ensatina per plot relative to terrestrial habitat variables in the upper Sacramento River in winter 1991 and spring 1992. Single variables with $P < 0.15$ and the best multivariate model are given. C is the sign of the regression coefficient..

Variable	C	Adjusted r^2	F	P
Winter 1991 survey				
Elevation	-	0.519	32.35	<0.0001
Log(trees >80 cm DBH)	+	0.268	11.63	0.002
Log(hardwoods >50 cm DBH)	+	0.189	7.76	0.010
Downed wood >10 cm DBH	+	0.106	4.43	0.044
Rock cover class	+	0.056	2.73	0.110
Elevation, log(trees >80 cm DBH), downed wood	-,+,+	0.665	17.20	<0.0001
Spring 1992 survey				
Elevation	-	0.325	14.93	0.001
Log(hardwoods >50 cm DBH)	+	0.199	8.19	0.008
Trees > 80 cm DBH	+	0.180	7.35	0.011
Downed wood > 10 cm DBH	+	0.049	2.51	0.125
Elevation, trees > 80 cm DBH	-,+	0.381	7.21	<0.005

Aquatic Surveys

The metam sodium spill appeared to decrease amphibian populations on the aquatic stations. Both average species richness and the frequency of occurrence of Pacific giant salamanders were generally higher on reference tributaries and rivers than on the Sacramento River. Combined with the observation of dead salamanders on river banks after the spill, these data are strong evidence of a negative impact.

Determining the extent of these impacts depends on accurate predictions of pre-spill abundance. Correlations between salamander abundance and habitat variables (Parker 1991, Welsh[3] 1993) from reference sites can be used to predict the abundance of a species in an area where pre-impact data are not available. For Pacific giant salamanders, habitat-abundance relationships suggested that 1.4 salamanders per plot lived in the Sacramento River before the spill. If this prediction is correct, populations in the Sacramento River may still be as much as 93% lower than pre-spill populations.

Although abundance-habitat relationships are a useful tool for predicting pre-spill populations, we have two caveats. Our regression equation accounted for only 23% of the variation in the number of animals per plot and was based on only 1 habitat variable. In addition, increased predation on the tributaries after the spill may have altered abundance-habitat relationships. These problems could have been resolved by measuring habitat variables on more reference sites. Habitat surveys, however, are expensive and funds were unavailable for these surveys.

Three years after the spill, we were unable to detect recovery in amphibian species richness or Pacific giant salamander abundance. The first salamanders found in the Sacramento River were five 1st-year larvae on a single plot near the mouth of Mears Creek in 1992. By 1993, 1st-, 2nd-, and 3rd-year Pacific giant salamander larvae were

found at the Soda River, Conant, Sims, Gibson, and La Moine stations, suggesting that recolonization had begun. However, in 1994, we found animals on only 2 plots: Sims and Gibson.

Long-term monitoring can provide evidence of environmental impact and its extent. However, recovery can be slow or sporadic and obtaining sufficient data requires a long-term commitment. In this study, we were unable to detect measurable recovery in Pacific giant salamander populations in 3 years. Although this time period may be long relative to litigation procedures regarding the spill, it is short relative to Pacific giant salamander colonization and population growth rates.

Terrestrial Surveys

We detected no impacts to amphibians on terrestrial plots. Species richness was not significantly lower on the Sacramento River than on tributaries and ensatina abundance was actually greater on the Sacramento River than on the tributaries.

A pattern of increasing ensatina abundance with distance from the spill site initially appeared to be related to the spill. However, abundance-habitat relationships indicated that this pattern could not be attributed solely to the spill. Sixty-seven percent of the variation in ensatina winter abundance and 38% of the variation in spring abundance could be attributed to elevation, number of large trees, and area of downed logs found on the plots. These habitat variables have been shown to be correlated with the ensatina abundance (all 3 variables) and amphibian species richness (elevation) in other studies (Aubry and Hall[4] 1991, Bury et al.[5] 1991, Corn and Bury[6] 1991, Welsh and Lind[8] 1991).

Our inability to determine an impact to ensatina or other species on terrestrial plots highlights a problem inherent to impact studies. If gradients in environmental perturbations are highly correlated with habitat variables that determine species distribution or abundance patterns, it is difficult to detect impacts. In this study, elevation and distance from the spill site (which is possibly related to herbicide by-product exposure) are highly correlated ($r = 0.99$). Thus, it is not possible to distinguish the relative effects of these variables on amphibian populations.

Suitability of Reference Sites

Efforts to assess impacts of environmentally harmful events are usually hampered by the lack of pre-disturbance data. Although these data are necessary to address impacts definitively, other methods, such as comparison of affected areas with reference sites, can provide a reasonable assessment of whether an impact occurred and its extent. The choice of reference sites, however, must be evaluated carefully.

Intensive habitat surveys and plot choice criteria suggest that the reference sites on the tributaries in this study were appropriate. Tributary reference sites were similar to the impacted Sacramento River sites in 8 aquatic and 8 terrestrial habitat features important for the 2 most abundant species: Pacific giant salamander and ensatina. Due to funding limitation, habitat surveys could not be conducted on the 3 additional aquatic reference sites (Trinity, Salmon, and Smith rivers) used to

evaluate impacts to Pacific giant salamanders. However, the criterion for choosing those sites was the presence of unembedded cobble substrate, a feature that dominates the Sacramento River impact area and is the best predictor of Pacific giant salamander density (Parker 1991).

The Smith River had particularly high densities of Pacific giant salamanders. Unlike the Trinity, Salmon, or Sacramento rivers, the Smith River does not experience extreme seasonal fluctuations in water temperature caused by snow melt and could support higher densities of Pacific giant salamanders. However, despite the high numbers of animals found on the Smith River plots, the frequency of plots with Pacific giant salamanders was not higher than that found on the Trinity River. Thus, we considered the Smith River an appropriate reference site for this study.

Three lines of evidence suggest that the aquatic reference sites may have underestimated the extent of the impact on amphibians. First, plot width and slope were significantly different between the tributaries and the Sacramento River. Aquatic habitats with narrower stream width and steeper slope, such as that found on the tributaries, have been found to support fewer animals (Welsh[3] 1993).

Second, anecdotal observations of river otters, *Lutra canadensis*, foraging on tributaries suggested that predators moved to tributaries when food supplies were extirpated from the Sacramento River. Up to 24% of river otter diet is comprised of Pacific giant salamanders (Reeves[11] 1988). During terrestrial and aquatic surveys in November and December 1991, we saw 4 river otters on tributaries (Boulder, Soda, and Hazel creeks) and 1 upstream of the spill site at the mouth of Ney Springs Creek. An autopsy of a dead otter found in Hazel Creek in December 1991 revealed that it died of starvation (D. Smith, CDFG, personal communication). These observations underscore the need to identify reference sites that are not likely to be influenced by indirect effects of the environmental impact being studied.

Third, at 1 of the Salmon River stations, we found only 1 Pacific giant salamander. Although the site appeared to be pristine, a gold dredge mine was located <3 km upstream of the station and may have had ongoing or historic impacts on salamander populations. Although we attempted to locate our reference sites in pristine areas, finding unimpacted riverine habitat was difficult. Most large watercourses in California have been impacted by dams, mines, or other developments that increase siltation of the substrate.

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[11] Reeves, K.A. 1988. Summer diet and status of river otters on Redwood Creek. M.S. Thesis, Humboldt State University, Arcata, California, USA.

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POSSIBLE IMPACTS OF THE CANTARA SPILL ON REPTILE POPULATIONS ALONG THE UPPER SACRAMENTO RIVER

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We assessed damage to reptile populations on the upper Sacramento River 1 year after a herbicide spill. In areas with >50% canopy cover, species richness was significantly lower and leaf-litter burrowers and semi-aquatic species were under-represented on plots with high vegetation-injury values. Abundance of western fence lizards, *Sceloporus occidentalis*, was 70% lower on dense canopy plots with high vegetation-injury values. A decrease in weight:snout-vent length ratio of male western fence lizards and an increase in fence lizard parasite load with distance from spill site did not appear to be caused by the spill. Garter snakes, *Thamnophis* spp., were under-represented on open canopy plots below the spill site.

INTRODUCTION

On 14 July 1991, 72,000 liters of the herbicide metam sodium accidentally spilled from a train car into the upper Sacramento River at the Cantara Loop. As the 16-km aqueous plume of herbicide traveled downriver, airborne by-products traveled low over the ground leaving residues in the soil (CDFG[1] 1991). One turtle, 6 lizard, and 12 snake species were expected to occur near the river (Stebbins 1985) at the time of the spill (Table 1). The effects of the herbicide on reptiles had never been clinically determined and pre-spill data were not available to provide baseline information for post-spill comparative studies.

Natural history attributes may have determined the degree to which reptiles were exposed to metam sodium or its by-products. Semi-aquatic species, such as the western pond turtle, *Clemmys marmorata*, and garter snakes, *Thamnophis* spp., may have been directly exposed to herbicide in the water. Alligator lizards, *Elgaria* spp.;

[1]CDFG. 1991. Natural Resource Damage Assessment Plan, Sacramento River Cantara Spill, Shasta and Siskiyou counties. California Department of Fish and Game, Redding, California, USA

Table 1. Reptile species expected to occur in the Cantara Spill area of the upper Sacramento River and natural history characteristics that may have affected their risk of exposure to metam sodium and its by–products. At the time of the spill in July 1991, all species were active. + = exhibits characteristic, – = does not exhibit characteristic.

Species	Semi-aquatic	Burrows extensively in leaf litter
Western pond turtle, *Clemmys marmorata*	+	–
Western fence lizard, *Sceloporus occidentalis*	–	–
Sagebrush lizard, *Sceloporus graciosus*	–	–
Western skink, *Eumeces skiltonianus*	–	+
Western whiptail, *Cnemidophorus tigris*	–	+
Southern alligator lizard, *Elgaria multicarinata*	–	+
Northern alligator lizard, *Elgaria coerulea*	–	+
Rubber boa, *Charina bottae*	–	+
Ringneck snake, *Diadophis punctatus*	–	+
Sharp-tailed snake, *Contia tenuis*	–	+
Striped racer, *Masticophis lateralis*	–	–
Racer, *Coluber constrictor*	–	–
Gopher snake, *Pituophis melanoleucus*	–	–
California mountain kingsnake, *Lampropeltis zonata*	–	–
Common kingsnake, *Lampropeltis getula*	–	–
Common garter snake, *Thamnophis sirtalis*	+	–
Western terrestrial garter snake, *Thamnophis elegans*	+	–
Western aquatic garter snake, *Thamnophis atratus*	+	–
Western rattlesnake, *Crotalus viridis*	–	–

western skinks, *Eumeces skiltonianus*; and other species that spend significant amounts of time burrowing through leaf litter may have received the highest exposure to residues (CDFG[1] 1991). Many species also may have been indirectly affected by post-spill changes in predator or prey populations.

Quantitative measurements of the duration and intensity of habitat exposure to the herbicide and its by-products are not available. A principal components analysis of vegetation-injury data is the best available data for habitat exposure (Willits[2] 1994). This analysis was based on data collected by Department of Fish and Game (CDFG) personnel in September 1991 from randomly placed transects in riparian vegetation in and above the spill area. Variables associated with plant trauma symptoms (chlorosis, wilting, necrosis, defoliation, leaf death, plant death, and resprouting) on 6 "sentinel" species (4 woody and 2 herbaceous) were recorded. The first principal component (based on plant death, resprouting, defoliation, chlorosis, necrosis, and leaf death) value was standardized for the relative extent of habitat types (riparian scrub, riparian forest, gravel bar, and upland) and the resulting

[2] Willits, N.H. 1994. Statistical consulting/programming report: Cantara data analysis quarterly summary. Report to California Department of Fish and Game, Redding, California, USA.

least square mean (LSM) values, relative measures of the spill's impact on each transect, indicated that plant damage was patchy below the spill site.

The purpose of this study was to determine whether and to what extent reptiles were affected by the herbicide spill by comparing reptile populations in areas with low and high vegetation injury.

METHODS

Plot Selection

We established 30 reptile survey plots overlapping the vegetation-injury transects. Plots were not placed on transects that: 1) showed impact by humans, such as campsites and dwellings; 2) were too steep to search safely for reptiles; 3) were <300 m from a plot that had already been established; or 4) overlapped terrestrial amphibian survey plots used in other studies (Luke and Sterner 2000). The survey plots ranged from 9 to 55 km below and 0.9 to 9 km above the spill site (Fig. 1). Plots could not be placed in the area 0–8.9 km below the spill site without overlapping terrestrial survey plots used in amphibian studies. Plots were 28 x 180 m with the length running parallel and immediately adjacent to the river. Reptile survey plots were not placed on vegetation-injury meanders on Campbell and Soda creeks that were surveyed by the CDFG to obtain estimates of vegetation-injury in low impact areas (Fig. 1). These areas were not immediately adjacent to the creeks and reptile habitat appeared to be substantially different from the rest of the plots.

Surveys

A team of 8 biologists conducted terrestrial and shoreline surveys for reptiles on all 30 plots. Terrestrial surveys were conducted from 28 May to 4 June 1992 and 24 to 26 June 1992. To insure complete coverage of a plot, each plot was subdivided into 4 parallel 7 x 180-m subsections. Four surveyors, 1 per subsection, searched for reptiles for 1 hour. Time spent capturing and processing reptiles was not included in total search time. The survey was divided into 2 phases. The initial phase was a quiet search, with minimal noise and disturbance, for active animals in the plot. All animals captured during this phase were marked with white correction fluid to insure that they were not counted again. During the 2nd phase, we backtracked over the section we had just surveyed, searching for inactive reptiles under cover objects such as rocks, logs, leaf and needle litter, and man-made materials. Surveyors searched under each type of cover with equal intensity. Marked animals were ignored during the 2nd phase. Throughout the field surveys, surveyors were rotated among above-spill and below-spill plots and reassigned to different teams to minimize the effect of surveyor bias.

Shoreline surveys were conducted on all 30 plots between 22 and 27 June 1992. One of the 8 surveyors first walked slowly along the bank for 30 minutes, stopping frequently to search with binoculars for reptiles. All reptiles observed were recorded and, if possible, captured. After completing this search, the surveyor used binoculars to observe the river for 15 additional minutes from a stationary observation point.

Figure 1. Location of reptile survey plots. Plots are named after the northernmost CDFG vegetation-injury transect that falls within its boundaries. Soda and Campbell creek meanders were used as reference areas by CDFG but were not sampled during reptile surveys.

During both terrestrial and shoreline surveys, all reptiles caught were identified, sexed, weighed, and measured. Mites and ticks (order Acarina) found on western fence lizards were counted and recorded. Habitat descriptions recorded at the location where each reptile was observed included: 1) distance from water; 2) presence of

riparian, ecotone, or upland vegetation; and 3) type of substrate and cover objects. Activity at the time of observation was recorded as moving or immobile in sun or shade, display/push-ups, courting, swimming, feeding, or mating.

Surveys were conducted only when air temperature was 21–32°C. After plot placement, we noticed wide variability in canopy cover, an important habitat variable in determining the distribution and abundance of reptiles. At the time of the survey, each plot was rated by surveyors as containing less or more than 50% canopy cover. The location of the plot was marked on a USGS topographic map and entered into a geographic information system (GIS). The GIS was used to measure the distance of the plot from the spill site.

Data Analysis

Data from terrestrial and shoreline surveys were evaluated relative to the CDFG vegetation-injury values. Least square mean values, from the principal component analysis of plant trauma symptoms on vegetation-injury transects (Willits[2] 1994), varied between −1.68 and 3.96 in the impact area. Least square mean values in reference areas (transects above Lake Siskiyou and on Campbell and Soda creek meanders) varied between −1.72 and 0.54 (Fig. 2). In all subsequent analyses, reptile plots with mean LSM values of ≤ 0.54 were defined as low vegetation-injury plots and those with mean values >0.54 were defined as high vegetation-injury plots. Mean vegetation-injury values were calculated for each plot by averaging the LSM values of all CDFG vegetation-injury transects ($n = 1-6$) that fell within plot boundaries.

Species richness was calculated as the number of species observed during both terrestrial and shoreline surveys on each plot. Abundance was calculated as the number of individuals captured on each plot during terrestrial surveys. Using only captured animals insured that individuals were not counted twice. Because western pond turtles were not observed or captured during terrestrial surveys, abundance for this species was calculated as the maximum number of individuals simultaneously observed on each plot during shoreline surveys. We used parasite load (the number of mites and ticks found on an individual) and the ratio between cube root of body weight in grams and snout–vent length (SVL) in millimeters as indicators of reptile health. Mean parasite load was calculated as the average number of parasites per individual on each plot. An average weight$^{1/3}$: SVL ratio was computed for each plot for use in subsequent analyses.

The small number of survey plots relative to the variance in species richness, abundance, and health variables resulted in very low statistical power. In preliminary analyses using 2- or 3-way ANOVA, the power of the test to detect effects of mean LSM values on the variables never exceeded 5%. Hence, we evaluated the data in a more qualitative manner.

Species richness, abundance, and health data from terrestrial surveys were first plotted against distance from spill and canopy cover to identify trends. Variables showing patterns that continued uninterrupted through the spill area and to above-spill plots were assumed to be unaffected by the spill. Variables that did not show patterns or that showed patterns relative to the spill area were then plotted against mean LSM

value and the relationships examined relative to high (>50%) and low (≤50%) canopy cover plots. A t-test was then used to find significant differences in measured variables between plots with high and low vegetation injury. Fisher's exact test was used to compare differences in the frequencies of plots with snakes, as a small value was observed in one of the cells. For these tests, $\alpha = 0.10$.

Before performing t-tests, dependent variables were analyzed for normality using a Shapiro-Wilk test (Zar 1984). Data not meeting normality were log transformed. In cases where log transformations did not result in a normal distribution of the data, we used a normal approximation of a Wilcoxon test to compare the means. Heterogeneity of variances was tested using a Bartlett test (Zar 1984).

RESULTS

The 30 reptile plots overlapped with 63 of the 132 vegetation-injury transects (Fig. 2). Five plots had high mean LSM values (>0.54) and 25 had low LSM values (≤0.54). All plots with high levels of vegetation injury had >50% canopy cover (Fig. 3a).

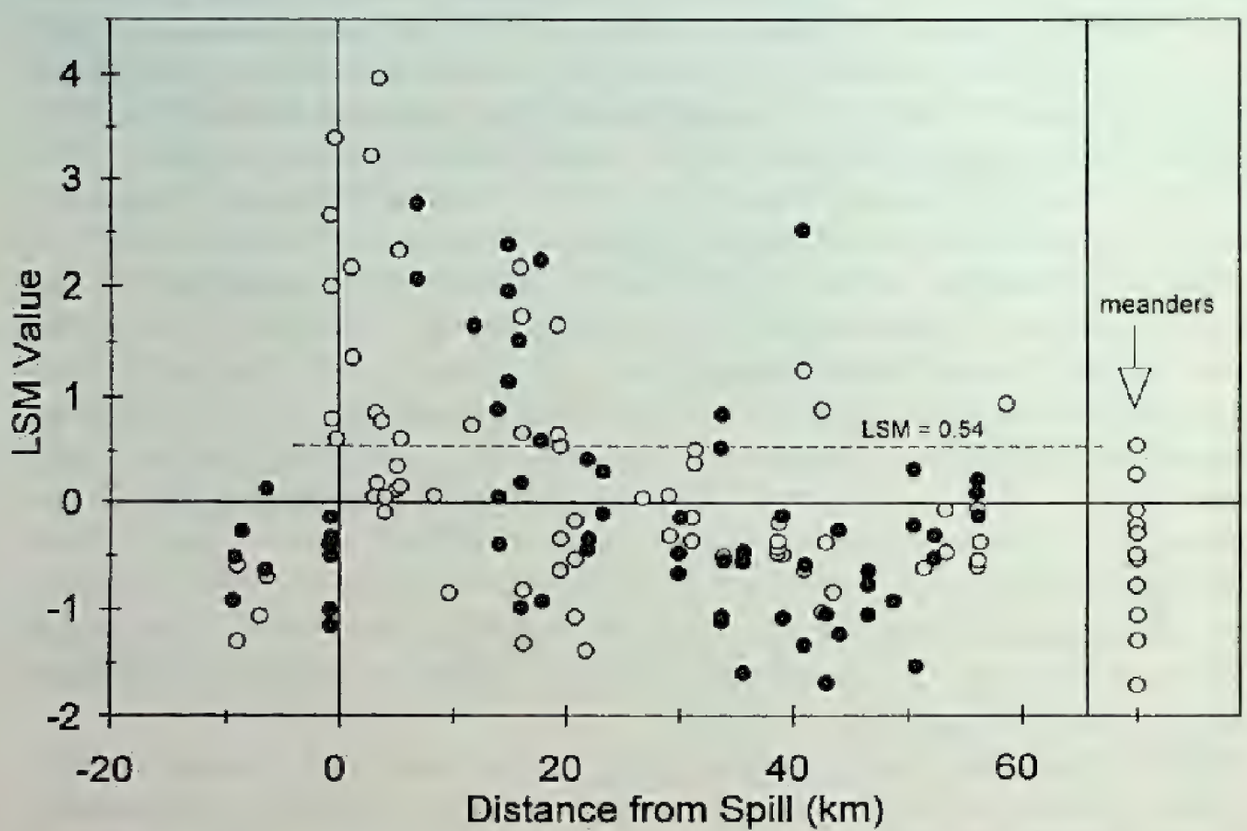

Figure 2. Least square mean values from 1st prinicipal component analysis of vegetation injury (Willets[2] 1994) plotted against transect location. Zero indicates the Cantara spill site. Values from control meanders are indicated to the right of the transects and are not plotted relative to their respective distances from the spill site. Filled circles are transects that fall within the boundaries of reptile survey plots. Least square mean values ≤0.54 are low vegetation-injury plots; values≥0.54 are high vegetation-injury plots.

Species Richness

Twelve of the 19 reptile species that occur in the region (Table 1) were found during terrestrial and shoreline surveys (Table 2). Shoreline surveys were more successful than terrestrial surveys at identifying semi-aquatic species; western pond turtles were only found during shoreline surveys, and a higher proportion of garter snakes was found during shoreline surveys than during terrestrial surveys.

The number of species per plot showed no geographic pattern relative to the spill area (Fig. 3b). When canopy cover was >50%, fewer species (mean = 1.2 species) tended to occur on plots with high vegetation injury than on plots with low injury (mean = 2.2 species; $t = 1.93$, $df = 14$, $P = 0.074$) (Fig. 4a). On plots with >50% canopy cover, leaf-burrowing and semi-aquatic reptiles (Table 1) tended to be found less frequently on plots with high vegetation-injury values (1-sided Fisher's $P = 0.094$).

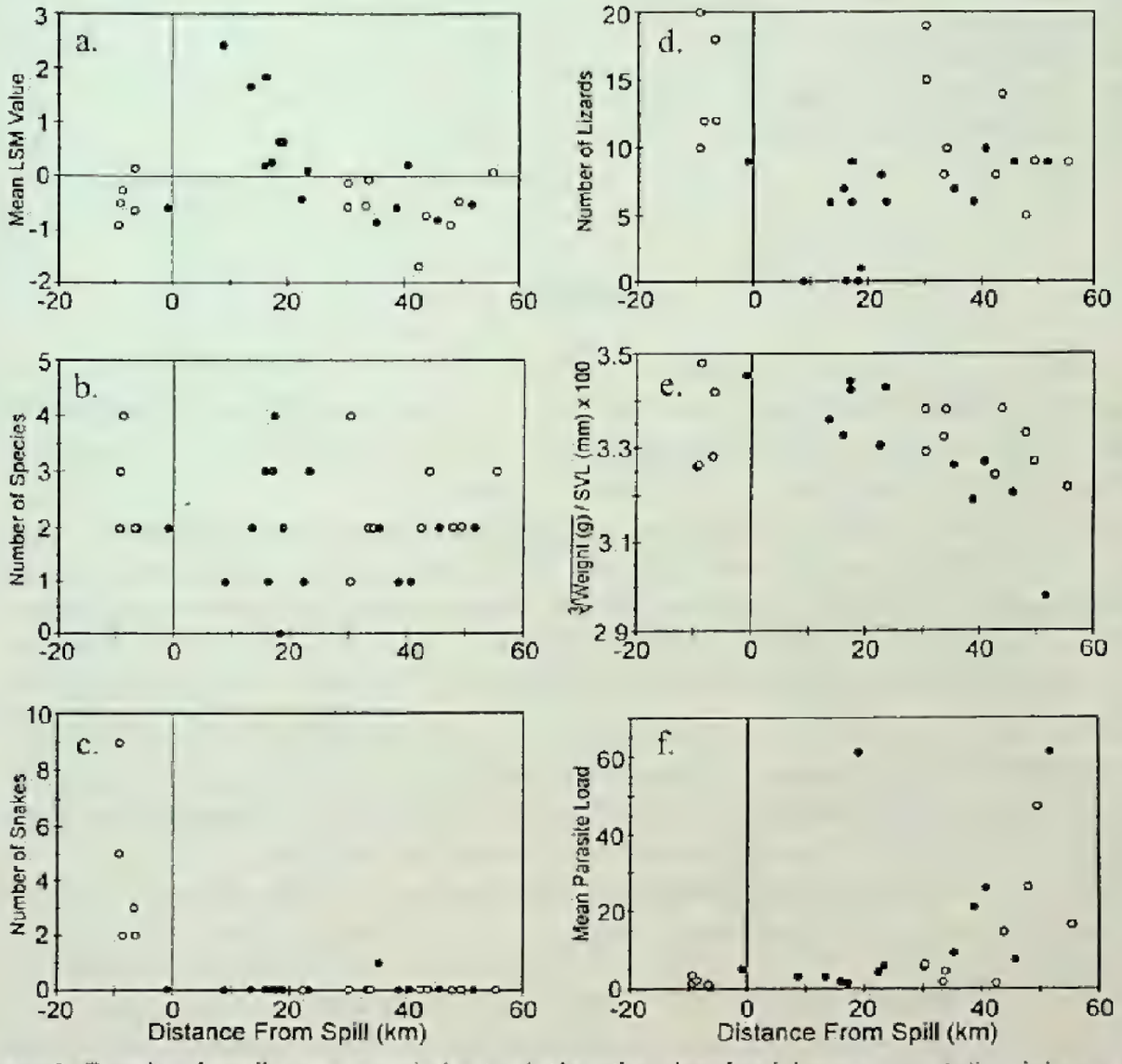

Figure 3. Results of reptile surveys relative to plot location showing (a) mean vegetation-injury value (least square mean from the 1st principal component [Willits[2] 1994]), (b) species richness, (c) garter snake abundance, (d) western fence lizard abundance, (e) mean weight$^{1/3}$: snout–vent length ratio for western fence lizards, and (f) mean parasite load for male western fence lizards. For all figures, closed circles represent >50% canopy cover and open circles ≤50% canopy cover.

Table 2. Reptiles found during terrestrial and shoreline surveys. For western pond turtles, "captures" were tallied as the number of individuals observed simultaneously on each plot.

	Terrestrial surveys		Shoreline surveys	
Species	Captures	% of total	Captures	% of total
Western pond turtle			5	22.7
Western fence lizard	511	85.2	5	22.7
Sagebrush lizard	46	7.7	1	4.5
Western skink	2	0.3		
Western whiptail	1	0.2		
Southern alligator lizard	3	0.5	1	4.5
Northern alligator lizard	10	1.7		
Alligator lizard, unidentified species	1	0.2		
Ringneck snake	1	0.2		
Racer	2	0.3		
Common kingsnake	1	0.2		
Common garter snake	1	0.2		
Western terrestrial garter snake	20	3.3	10	45.5
Garter snake, unidentified species	1	0.2		
Total captures	600		22	

Abundance

A total of 600 reptiles was captured during terrestrial surveys (Table 2). The western fence lizard was the most abundant species, representing 85% of the terrestrial captures. The western terrestrial garter snake, *Thamnophis elegans*, was the most abundant snake, representing 3% of reptiles captured.

Garter snakes were found on 6 plots, all with low vegetation-injury values, and all but 1 with ≤50% canopy cover (Fig. 3a, c). On plots with ≤50% canopy cover ($n = 14$), garter snakes were found more frequently above (5 of 5 plots) than below the spill site (0 of 9 plots, Fisher's $P = 0.0005$).

The number of western fence lizards declined on plots with >50% canopy cover within the first 30 km below the spill site (Fig. 3d) and abundance was significantly lower on high vegetation-injury plots (3.3 animals) than on low vegetation-injury plots (10.9 animals; Wilcoxon $z = -2.94$, $P = 0.0003$) (Fig. 4b). Abundance on plots with ≤50% canopy cover was high above and below the spill site.

Western pond turtles were found on 3 plots below the spill site only. All of these plots had low vegetation-injury values and >50% canopy cover.

Health Indicators for Western Fence Lizards

Both weight$^{1/3}$: SVL ratio and parasite load showed strong patterns that appeared to be independent of the spill site (Figs. 3e and 3f). Mean weight1/3: SVL ratio of male lizards was highest above the spill and gradually decreased with distance below the spill site. No pattern was observed for female weight1/3: SVL ratios. Mean

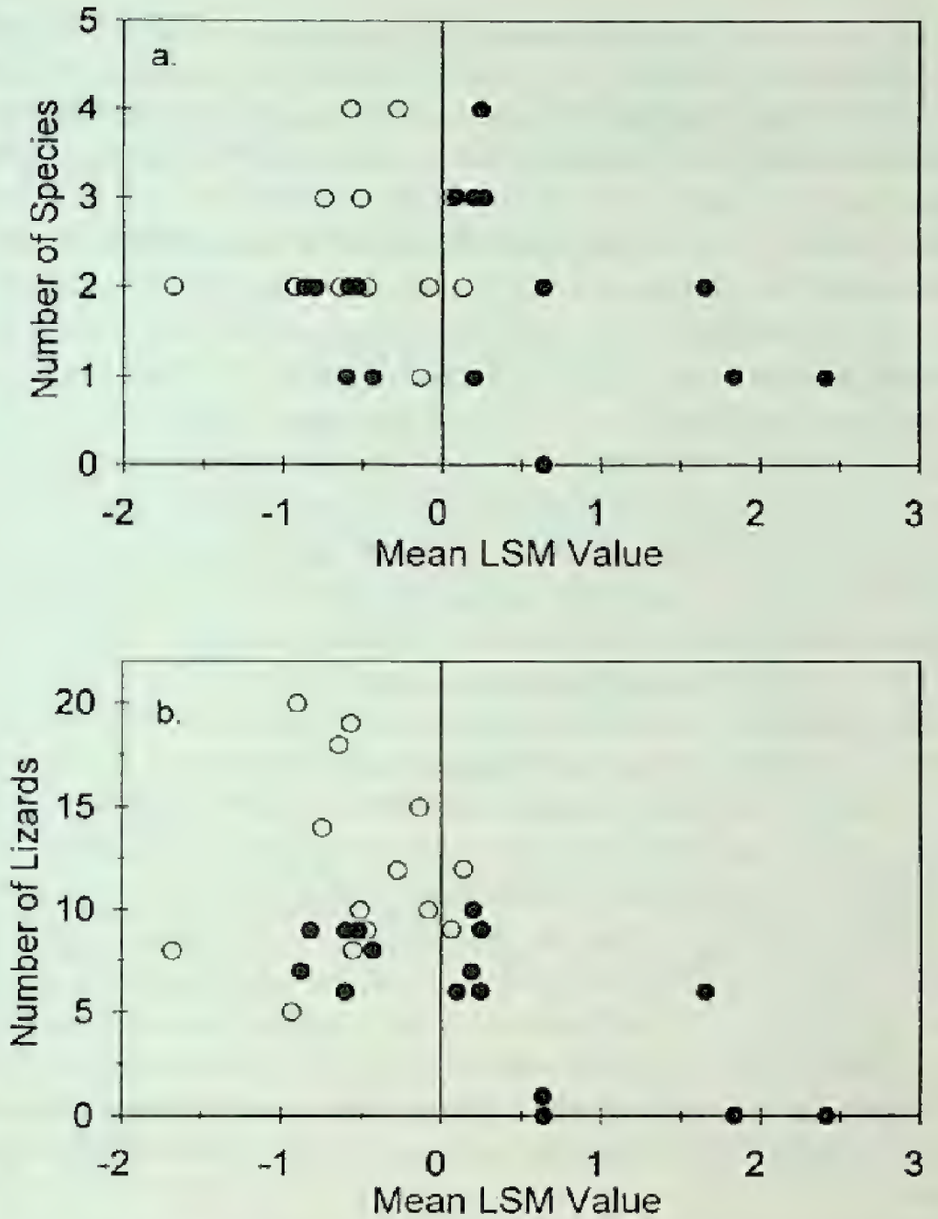

Figure 4. Species richness (a) and mean western fence lizard abundance (b) relative to mean vegetation-injury (LSM) values after the Cantara Spill on the Sacramento River. Closed circles represent >50% canopy cover and open circles ≤50% canopy cover.

parasite load was lowest above the spill site, remained low (<10 parasites/individual) to about 35 km below the spill site and then suddenly began increasing with the highest values (47–61 parasites/individual) at 50 km below the spill site.

DISCUSSION

Ideally, impacts of the metam sodium spill on reptiles would be quantified by comparing pre- and post-spill data on the abundance of each species in the impact area. Unfortunately, because pre-spill data on reptile populations are not available, post-spill patterns in reptile populations cannot be unequivocally attributed to the herbicide. In the absence of such data, we addressed potential effects of the spill by examining associations between a post-spill vegetation-injury index and reptile species richness, abundance, and health variables. While the approach has assumptions that

are difficult to test (i.e., high vegetation-injury values were caused by the spill, low vegetation-injury values are similar to pre-spill values, and reptiles in areas with higher levels of vegetation damage were exposed to higher levels of metam sodium or its by-products), it provides evidence for potential impacts and recommendations for further study.

Post-spill patterns in species richness and abundance suggest that reptiles were impacted by the herbicide spill in areas with dense canopy cover (Fig. 4a, b). Fewer species (45%, 1 species less) on plots with high vegetation-injury values suggests that reptile populations declined in areas subjected to high levels of herbicide exposure. In addition, the tendency for leaf-litter burrowing and semi-aquatic species (Table 1) to be absent from high impact plots suggests that these species may have experienced greater population declines due to higher levels of exposure to the herbicide.

A similar pattern was found in the abundance of western fence lizards in areas with dense canopy cover; plots with high levels of vegetation injury supported 70% fewer fence lizards than plots with low levels of vegetation injury. This observation suggests that even populations of common species that show no particular susceptibility to the herbicide may have declined due to herbicide exposure or other spill-related impacts. Health of western fence lizards, however, did not appear to be affected by the spill. Weight:SVL ratio reflects the ability of lizards to find and consume insect prey that may have been affected by the spill. This ratio decreased with increasing distance downstream. Parasite load, which may imply a cost to lizards or reflect their ability to reject parasitic colonizations, increased with distance downstream. Both of these health indicators showed no abrupt changes at the spill site (Figs. 3e and 3f) and were probably related to variation in other habitat variables. Many habitat variables known to affect reptile densities (Carpenter 1952, Fleharty 1967, Marcellini and Mackey 1970, Rose 1976), such as elevation, temperature, or rainfall, vary clinally in the study area and may be directly or indirectly responsible for these patterns.

Because all open-canopy plots occurred in areas with low vegetation-damage indices, we could not make comparisons on open-canopy plots between reptile populations in areas with low and high vegetation-damage indices. Our study therefore cannot address impacts to reptile populations in open-canopy areas where many species are most abundant.

Our study provides preliminary evidence that reptile populations were impacted by the Cantara Spill. We recommend 2 additional studies to provide more conclusive evidence for impact and its extent. First, reptile populations on plots with dense canopy cover should be monitored over time. Increases in species richness and abundance on plots with high levels of vegetation injury, but not on those with low vegetation injury, would provide more conclusive evidence that the observed trends are related to spill impacts. Second, to insure that observed trends are not caused by habitat variation, detailed measurements of habitat variables and how they correlate to species abundance should be documented. For example, the Sacramento River above Lake Siskiyou appeared to be shallower and warmer than below the lake and this could explain the greater abundance of garter snakes found upstream of the spill.

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RESTORATION PLANNING FOR THE CANTARA METAM SODIUM SPILL: A GROUP MULTIATTRIBUTE DECISION ANALYSIS APPROACH

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A US$38 million settlement with Southern Pacific Transportation Company makes more than $14 million available to help restore natural resources injured by the 1991 release of metam sodium into the upper Sacramento River. A group multiattribute decision analysis approach was selected and adapted to help natural resource trustees in evaluating potential restoration projects. Initially used to screen urgent projects, it is now used annually to help solicit and evaluate additional projects. Planning objectives focused on maximizing the project's expected benefits and relationship to the injuries, while also providing consistency with settlement agreements and resource policies of the trustee agencies. To establish a framework for evaluating grant proposals over a 5-year period, an interdisciplinary team of specialists knowledgeable about the injuries and site conditions identified general restoration requirements, determined project categories, developed selection criteria and rating scales, and assigned initial criteria weights. This framework was incorporated as technical guidance in an annual solicitation for restoration proposals and used as a tool to evaluate proposed projects. A technical panel ranked proposals, commented on cost completeness and realism, and made recommendations to help Trustees make final selections. The decision analysis approach has: 1) focused projects on high priority requirements; 2) provided a clear, efficient, documented, and defensible approach for group evaluation of projects; and 3) enabled trustees to expedite and adaptively manage the ongoing restoration planning.

INTRODUCTION

The Cantara Trustee Council (Council) was challenged to oversee the expenditure of more than US$14 million for natural resource restoration received as part of the $38 million settlement from Southern Pacific Transportation Company following the 1991 spill of metam sodium into the upper Sacramento River. Under terms of the Memorandum of Agreement (MOA) implementing the settlement, restoration expenditures were used for 1) resource protection, restoration, rehabilitation, and enhancement; 2) acquisition, study and/or research; and 3) program support (CDFG[1] 1994). The trustee agencies acted immediately to prevent further injury and begin resource restoration. The Council solicited project proposals for additional restoration and evaluated the relative merit of these proposals using a group decision analysis method.

We describe a general decision analysis approach and specific methods that were used to evaluate proposals submitted for funding from the Upper Sacramento River Account (Account) established to hold the restoration settlement funds. The Council-designated Technical Review Panel (Panel) composed of members representing all the trustees has been using this method for 3 years to provide administrative support for the Council. The Council considered the Panel's recommendations in their annual decisions on how the Account's funds were expended.

The decision analysis approach was tailored for this application by the Cantara Program Team (Team) consisting of members of the California Department of Fish and Game's (CDFG) Cantara Program and Legal Office, with the assistance of a consulting analyst. The CDFG units were responsible for overseeing the injury and damage assessments for the Cantara Spill as well as the initial restoration activities carried out to respond to the natural resource injuries resulting from the spill. To expedite restoration implementation, the approach and evaluation system were developed before the settlement was reached and the Council appointed.

DECISION SUPPORT GOALS

The overall goal of the decision analysis approach was to provide an effective, rational, and appropriate method for evaluating proposals to ensure that the evaluation process was 1) consistent with the Trustee values and preferences outlined in the MOA, the governing Department of Interior (DOI) Natural Resource Damage Assessment (NRDA) Regulations, and related agency policy; 2) impartial, well documented, and defensible; 3) appropriate for the current restoration requirements; and 4) flexible, to allow adjustments over time as program requirements or Council priorities changed.

Decision analysis was applied as a means to provide a consistent method for the technical review of proposals based on objectives and criteria approved by the Council.

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[1] CDFG, 1994. Memorandum of Agreement executed as part of the consolidated actions entitled "People of the State of California, ex. Rel. Douglas Wheeler, Sec. of the Resources Agency et al. V. Southern Pacific Transportation Company, Inc." Case no. CIV S-92-2090-WBS-GGH.

Although the Panel's recommendations informed the Council, final funding decisions on restoration projects were made by the Council.

DECISION ANALYSIS APPROACH

Effective restoration planning requires clear objectives and the development of criteria by which their achievement can be reliably measured. To accomplish this, it is necessary to integrate information about trustee values, baseline resource conditions, the nature and extent of injuries to natural resources and the services resources provide, and the recovery rates or the results of direct restoration projects already implemented. In addition, the feasibility of proposed restoration projects and the potential for collateral injury or benefit must be assessed. Simply identifying a set of alternatives via group discussion or brainstorming methods does not necessarily provide a rational, effective, and defensible program to restore resources or encourage innovative approaches.

The decision analysis approach for Cantara restoration planning was designed to assist the interdisciplinary Cantara Program Team in identifying requirements, establishing priorities, and then developing a systematic approach for evaluating proposals. It was a structured systems approach (sensu Churchman 1968) adapted from well-established methods (Keeney and Raiffa 1976) applied successfully for a range of similar types of decisions.

The basic decision analysis approach used 9 steps:

1) Structure the problem based on Trustee values and preferences (sensu Keeney 1992).

2) Identify objectives and organize projects into categories.

3) Decompose and model the decision by establishing selection criteria and rating scales, that reflect the Trustees' preferences.

4) Establish the expected range of potential scores and assign initial criteria weights.

5) Validate the method using test projects and sensitivity analyses.

6) Prepare solicitations or requests for proposals focused on the established objectives and criteria.

7) Review and recommend appropriate projects to the Council and document these recommendations for efficient and effective review.

8) Adaptively modify the decision process as required to meet changes in restoration requirements (from feedback on natural recovery, impact of other projects, etc.).

9) Document the lessons learned and the general performance of the method for future applications.

CANTARA PROGRAM APPLICATION

The general decision analysis approach outlined above was adapted for the Cantara restoration program. This involved selecting the factors to consider, the specific decision model to be used, and establishing evaluation parameters that reflected Trustee values.

Structure the Problem

The Trustees intended that, to the extent practicable, restoration funds should be spent according to Trustee priorities included in the MOA and guidance from the governing regulations. The 1st step in the decision analysis approach was designed to frame the working problem and characterize Trustee values.

To provide a framework for additional restoration that would encourage the development of a broader portfolio of projects, a value-focused (Keeney 1986, 1992) or attribute-focused (Carroll and Johnson 1991) approach was applied. The value-focused approach, as opposed to an alternative-focused approach, was based on clear functional restoration objectives and defined evaluation criteria. Functional objectives focus on what is to be achieved rather than on how it is achieved. This differed from an alternative-focused approach, frequently used in restoration and mitigation planning, where planners focus first on available restoration alternatives (how) rather than functional objectives (what). The value-focused approach ensured that conceptual alternatives did not get embedded in the objectives and thereby restrict the range of alternatives considered.

Identify Objectives and Project Categories

The next step in the analysis established a common set of objectives and classified potential projects into categories that were comparable in terms of their basic characteristics. The objectives were derived primarily from charter documents, trustee agency expert judgement, and current information on the state of injured resources. Charter documents included DOI's NRDA Regulations (DOI[2] 1991) and the Cantara MOA, as well as related trustee agency policy. Project categories were established to identify the types of projects solicited as outlined in the MOA and to achieve a common basis for evaluating projects. Objectives and categories were developed during a series of meetings involving the consulting analyst (D. Sheehy) and the CDFG Team members who were knowledgeable of the injuries, site conditions, and restoration requirements.

Department of Interior NRDA guidance identifies the following 10 evaluation factors that trustees should consider in selecting the means of restoration: 1) technical feasibility, 2) cost/benefit, 3) cost-effectiveness, 4) results of actual or planned responses, 5) potential for additional injury, 6) natural recovery period, 7) ability to recover with and without alternative action, 8) effects on health and safety, 9) consistency with relevant policies, 10) compliance with applicable laws. The regulations provide no guidance on how these incommensurate considerations should be combined or how tradeoffs might be made between competing factors. Multiattribute decision analysis provided a means for integrating these factors based on the specific values and preferences the Trustees expressed. How these factors

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[2] DOI (Department of Interior). 1991. Natural Resource Damage Assessment Regulations. 43 CFR Part 11. 56 Federal Register (19752), April 29, 1991.

were tailored to the Cantara restoration planning effort is outlined below. Details are provided in Sheehy, et al.[3] 1995.

Natural recovery period and the ability to recover with and without alternative action were considered screening factors that determined whether or not intervention was required. If a resource was expected to recover without additional action and the recovery period was short, only protection from further harm during recovery was required.

Consistency with relevant policies and compliance with applicable laws were also primarily screening factors. Attorneys from the CDFG Legal Office and the State Attorney General's office clarified legal constraints that might eliminate projects from further consideration. Policies of the CDFG and the U.S. Fish and Wildlife Service, related primarily to mitigation and endangered species concerns, were reviewed to ensure that potential inconsistencies were identified.

The results of actual or planned response actions were initially considered in the evaluation. However, since the contaminant of concern, metam sodium, was not persistent in the impacted environment and immediate human health and resource management concerns were already addressed, this factor was not directly relevant to subsequent restoration planning in this case.

The 2 primary cost-benefit and cost-effectiveness concerns were cost completeness and cost uncertainty. Cost completeness included consideration of all the costs incurred throughout the life of the project (Sheehy and Vik 1995), which included the Trustees' oversight, monitoring, and administrative costs. Cost uncertainty was considered to ensure that proposed projects could realistically be completed with the planned budget.

The remaining factors identified in the DOI NRDA Rule were technical feasibility, potential for additional injury, and effects on health and safety. The potential for additional injury and negative effects on health and safety were considered as a separate criterion, "collateral injuries" (Fig. 1), which negatively influenced a project's overall potential restoration benefit. Technical feasibility was included in a set of feasibility factors developed to provide a more realistic projection of expected benefit.

The MOA stated a preference for projects directly related to the nature and location of the injury and focused restoration, to the extent possible, on in-kind natural resources found on-site. This focus was also consistent with the mitigation policies of both California Fish and Game (CDFG 1987[4], 1990[5] and U.S. Fish and Wildlife Service (DOI[6] 1981).

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[3] Sheehy, D.J., P.B. Marshall, and N.H. Hammett. 1995. Cantara spill restoration planning: A decision support system for selecting restoration projects. Prepared for the California Department of Fish and Game, Redding, California, USA.

[4] CDFG. 1987. Department of Fish and Game recommended wetland definition, mitigation strategies, and habitat value assessment methodology. California Department of Fish and Game, Environmental Services Division, Sacramento, California USA.

[5] CDFG. 1990. Guidelines for establishment and use of wetland mitigation banks. California Department of Fish and Game, Sacramento, California, USA.

[6] DOI (Department of Interior). 1981. U.S. Fish and Wildlife Service Mitigation Policy. 46 Federal Register (7644-7633), January 23, 1981.

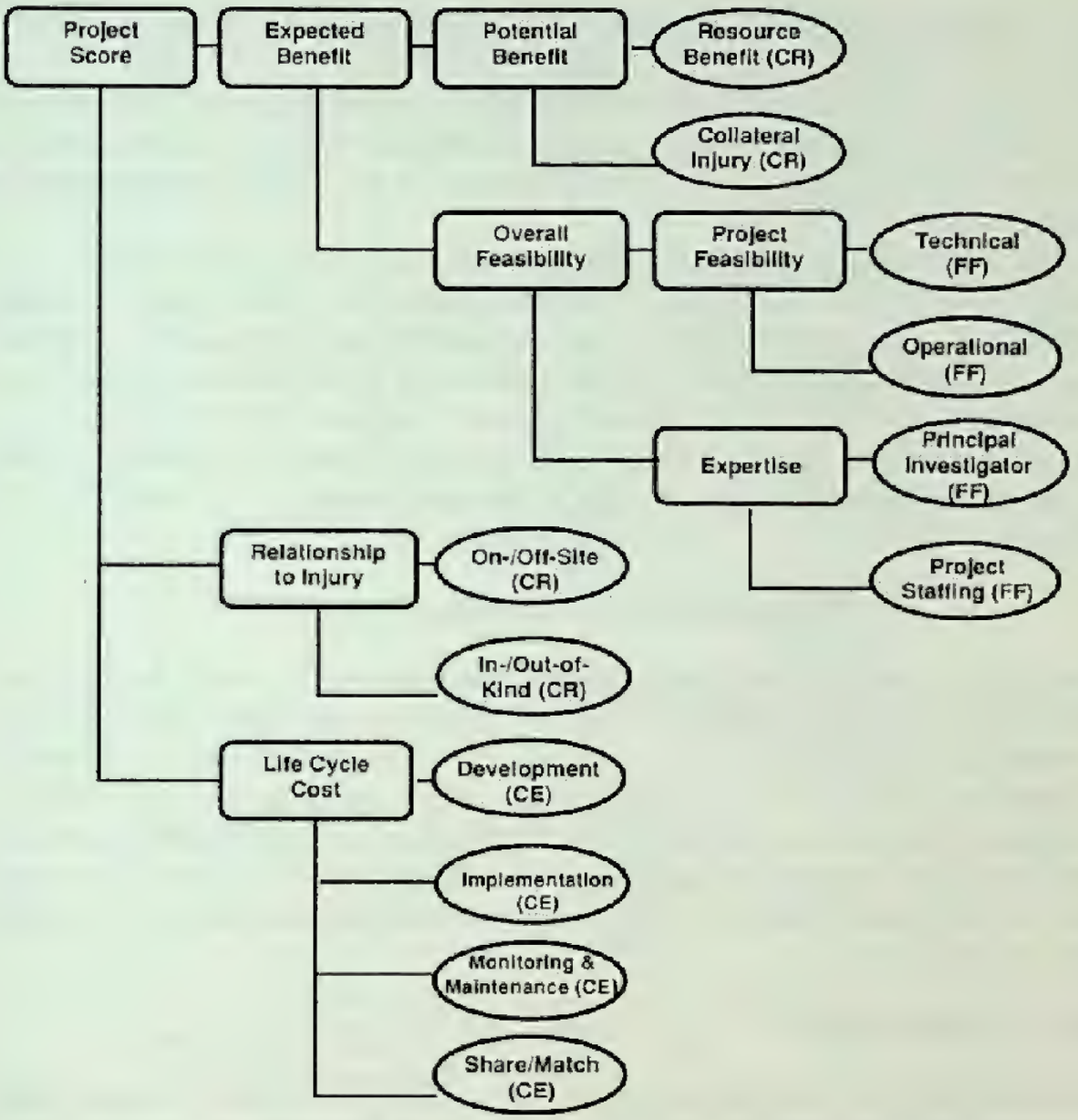

Figure 1. Value hierarchy for restoration, rehabilitation, and enhancement projects. Ovals indicate criteria (CR), feasibility factors (FF), or cost elements (CE) evaluated by review panel.

The analysis of the values and preferences in the DOI NRDA Regulations and the MOA and from focused discussions with the Team, led to the development of 3 overall objectives:

1) Provide the greatest practicable restoration benefit to the injured resources, communities, and habitats of concern.

2) Ensure that restoration activities were closely related to the resources injured.

3) Achieve the objectives cost-effectively and in a timely manner.

In addition, since the restoration was of considerable interest to other stakeholders with competing agendas and expectations, the approach used to select projects for funding also had to be clearly described; documented; and, if required, defensible.

Four categories of projects were identified as candidates for grant funding.

1) Restoration, rehabilitation, and enhancement projects restored injured resources to baseline conditions. These projects were to complement, but not

duplicate, projects already undertaken by CDFG and focus on those resources or habitats still in need of direct intervention.

2) Study and research projects were designed to enhance recovery work or assess new restoration methods (feasibility studies), document recovery, establish baseline conditions for other systems at risk, or contribute to the adaptive management of natural resources.

3) Acquisition and resource protection projects focused on acquisition of property, conservation easements, or resource rights that would serve to protect resources from further injury and maintain critical habitats or resources. Emphasis was on restoring special-status species, preserving desirable community types, providing landscape linkages, or providing new or improved public access.

4) Public information and education projects were developed to educate the public about the spill and promote and encourage the development of a stewardship ethic for these resources.

Decompose and Model the Problem

The primary objectives were decomposed into measurable criteria, rating scales, and feasibility factors in group discussions with the Team and trustee agency advisors. This process resulted in the development of a value hierarchy with objectives, criteria, and feasibility factors for each project category (Figs. 1–4) (Sheehy et al.[3] 1995).

Extensive Team discussion and interaction between the Team and the consulting analyst led to the iterative development of descriptive rating scales and associated numeric scores for each criterion. Rating scales included both the quantity and quality aspects of performance.

Additive Weighting Method

The simple additive weighting method, the best known and most commonly used compensatory multiattribute decision analysis model for evaluating natural areas, was selected as the basis for the decision support system. Additive weighting (Hwang and Yoon 1991) reflected a compromise between a straightforward and easy to understand evaluation method and more complex methods based on utility theory (von Neumann and Morgenstern 1947) that are more difficult to explain and implement. The additive model was selected because it provided a compensatory framework that allowed tradeoffs between incommensurate factors related to performance and relationship to injury, yet was simple to explain and apply without the complicated measurement and elicitation techniques required in more sophisticated compensatory models.

The additive weighting method can be applied when all the criteria are measured on an interval or ratio scale, all values are expressed in comparable units, and weights can be meaningfully assigned to each criterion. The method involves weighting the criteria, rating each alternative proposal for each of the selection criteria, converting these measures to a comparable form, and then multiplying all criterion values by their respective weights. Summing the resulting values provides a weighted total score used to rank each proposal in order of preference.

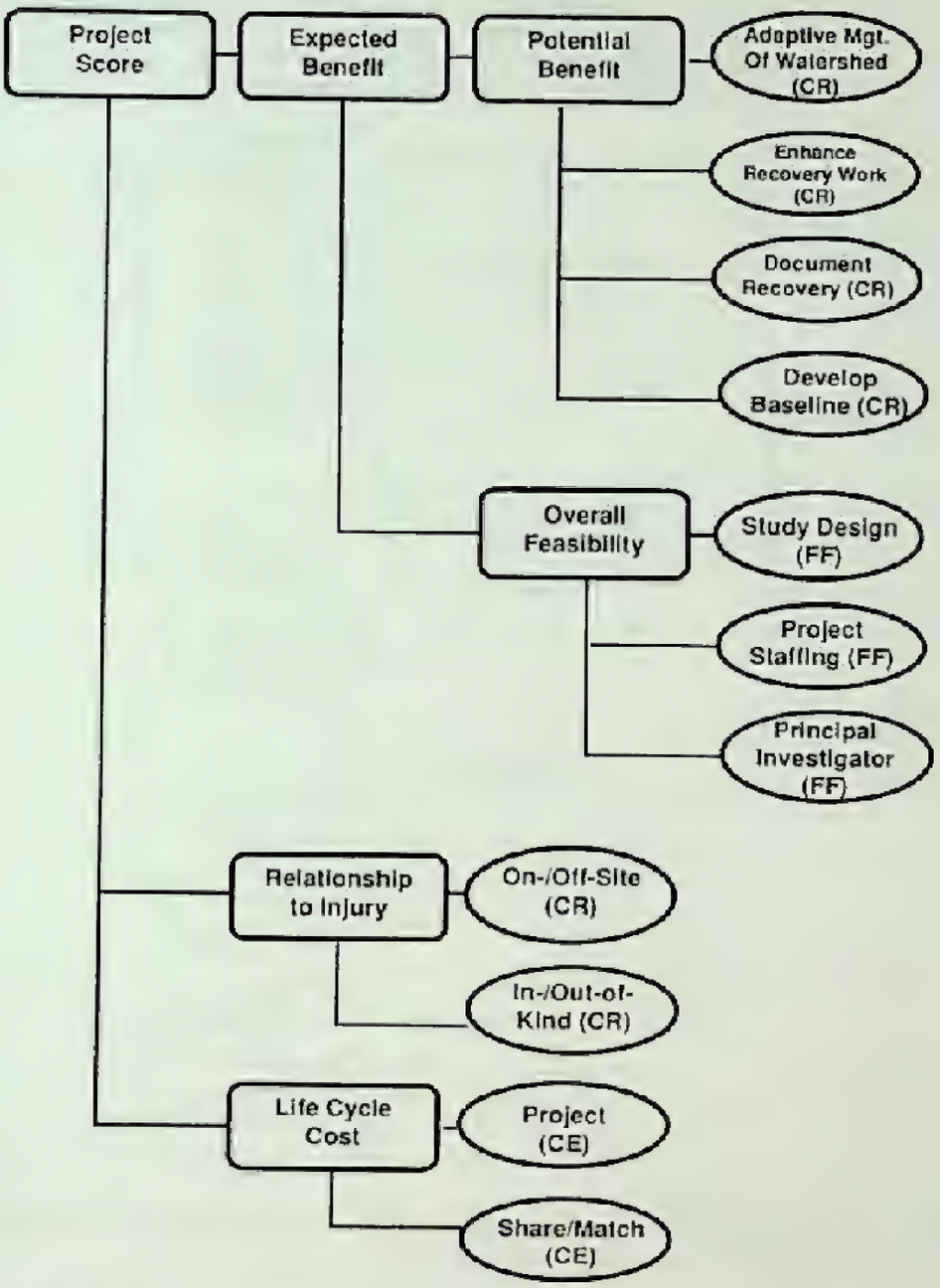

Figure 2. Value hierarchy for study and research projects. Ovals indicate criteria (CR), feasibility factors (FF), or cost elements (CE) evaluated by review panel.

Mathematically, the additive weighting method can be described as follows: Suppose the decision maker assigns a set of importance weights to criteria, $\underline{w} = \{w_1, w_2, \ldots, w_n\}$. Then the most preferred proposal, A*, is selected such that:

$$A^* = \{A_i \mid \max_i \sum_{j=1}^{n} w_j x_{ij} / \sum_{j=1}^{n} w_j \},$$

where x_{ij} is the outcome of the i^{th} alternative for the j^{th} criterion with a numerically comparable scale.

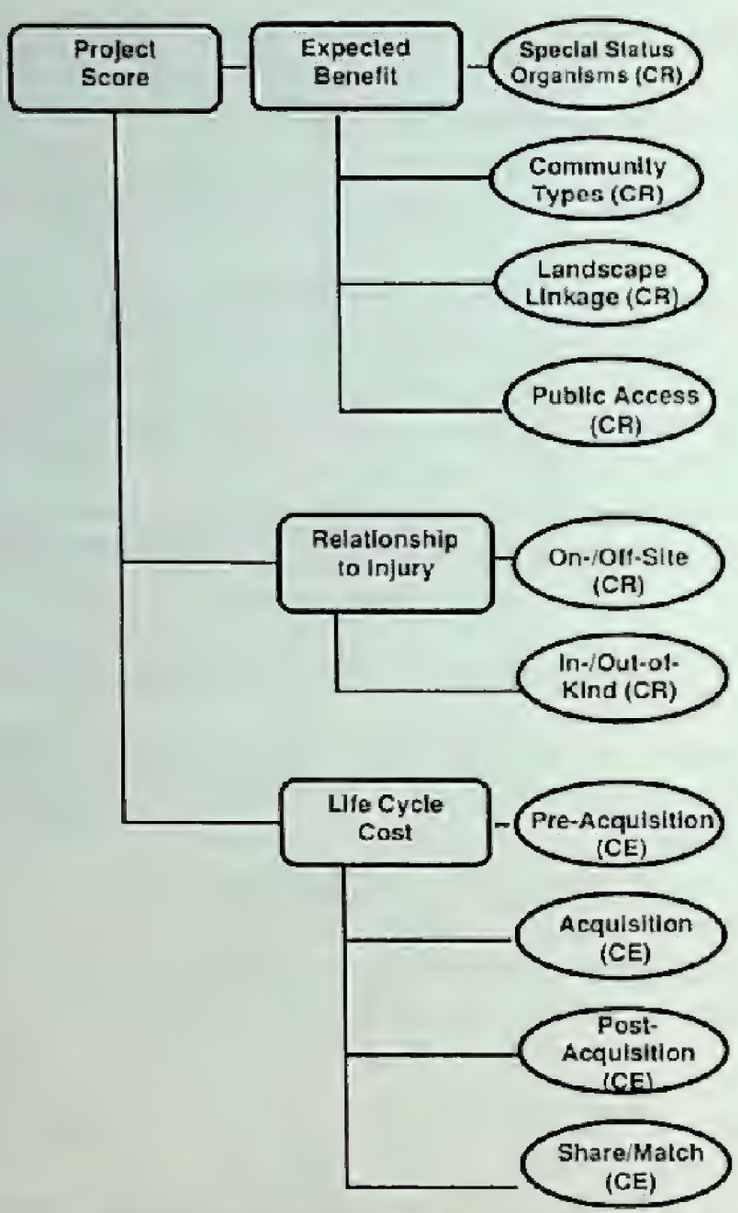

Figure 3. Value hierarchy for acquisition and resource protection projects. Ovals indicate criteria (CR) or cost elements (CE) evaluated by review panel.

Feasibility Factors

The simple additive weighting method was modified for this application by adding feasibility factors to provide a practical means of addressing performance uncertainty. Feasibility factors were developed by considering potential project failure modes (Sheehy and Vik 1995) and the nature of the project category. These feasibility components were combined by multiplying the weighted factors together and then normalizing them by dividing them by the product of the maximum scores. The overall feasibility was then multiplied by the project's potential benefit to predict its expected benefit, a more realistic estimate of project performance.

Feasibility factors were developed for all project categories except acquisition projects. Feasibility factors (shown in Fig. 1, 2, and 4) were related to technical and operational considerations for restoration projects or study design considerations for

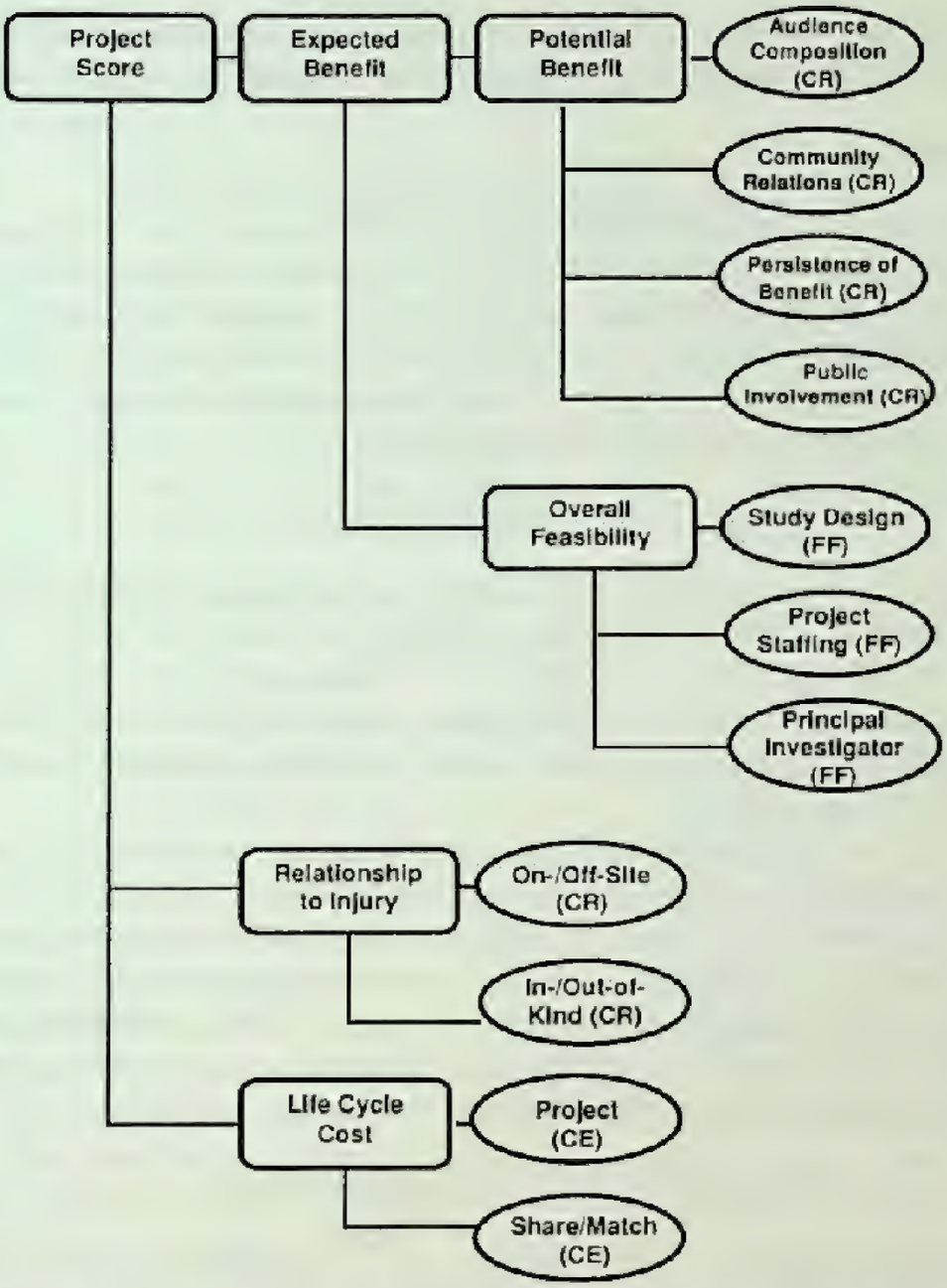

Figure 4. Value hierarchy for public information/education projects. Ovals indicate criteria (CR), feasibility factors (FF), or cost elements (CE) evaluated by review panel.

research or public information categories. Expertise and experience of the project participants were also considered as feasibility factors.

The use of feasibility factors is a modification to the standard additive weighting method. It can be argued as follows. The probability of success of a project depends on 1) the probability that the proposed project, as designed, can be implemented successfully by a qualified Principal Investigator (PI) and staff, 2) the probability that the proposing PI has the ability to implement this design, and 3) the probability that his or her staff has the ability to implement the design. The joint probability that all three components work successfully is the product of the individual probabilities. If any one of these probabilities is 0, then the joint probability is 0. This would

effectively disqualify a project from consideration since it would result in an expected benefit of 0 (J. S. Dyer, University of Texas, Austin, Texas, personal communication).

Software Support

A spreadsheet software program was developed to enable the Panel to compile annual proposal ratings according to the modified additive weighting model. This program allowed individual Panel member ratings to be recorded, compiled according to the modified additive weighting method, and combined to reflect a group preference structure for project alternatives. It was designed to allow rating scales and criteria weights to be changed during the 5-year program.

Weight Assignment

Weights are assigned to express the relative importance of different criteria for each project category. For this analysis, weights were assessed using a pairwise comparison or eigenvector method (Saaty 1977). During development, this approach was used to establish a set of initial preference weights for each Team member for each project category. It was also used to assign a weight to feasibility factors for all but the acquisition projects.

In applying this weighting method, the Panel member was asked to judge the relative importance of 2 attributes at a time. This approach was selected because it was easy to implement, provided repeatable results, and reduced biases associated with direct assignment methods. To reduce the number of pairwise comparisons required of Panel members, a slightly modified algorithm (Wilde and Beightler 1967), which also asked for strength of preference between pairs of criteria, was used. Weights were normalized to sum to 100 for ease of interpretation.

Test and Refine the Decision Model

There is an old saying in systems analysis that "all models are wrong, but some are useful." To determine the usefulness of the additive weighting model and to confirm that it captured the Team's preferences and values, a set of test proposals for each of the 4 project categories was identified and evaluated using the model. Once a set of projects was developed, criteria weights were assigned that reflected the criteria score ranges for the set of test projects. The Team then completed the evaluation process for each project category by assigning individual scores for criteria and probabilities for feasibility factors.

The evaluation results were used to adjust the model parameters until the Team felt that the results were consistent with their professional opinion of the relative merits of test proposals. Sensitivity analysis was used during system development and testing to identify which criteria, weights, or feasibility factors were driving the total scores and rank and to clarify where significant differences of opinion existed within the group. This insight stimulated discussions that helped the Team focus quickly on those issues that were important to the ranking and for which significant

differences in opinion remained. Testing resulted in the addition of a criterion, changes in criteria rating scales and weights, and adjustments to the spreadsheet software to make it easier to use.

Prepare Solicitation Technical Package

To communicate the requirements and the evaluation method to proposal originators, a solicitation package incorporating information on the decision analysis methods used in the evaluation was prepared. The grant program description included key aspects of the analysis. An appendix in the grant application instructions included details. The appendix explicitly described the project evaluation process and identified the information required for a complete grant proposal. It was revised annually to reflect changes in Trustee program requirements or the evaluation process.

Review and Recommend Appropriate Proposals for Trustee Council Consideration

The Technical Review Panel evaluated proposals received during 1996 and 1997 using the decision analysis methods to score and rank proposals and annotate those items that merited detailed attention by the Council (e.g., funding recommendations, completeness and realism of cost estimates, identification of data gaps or specific feasibility concerns, and the potential for interaction between proposals).

Each member of the Panel assigned criteria scores and feasibility probabilities to each project. Weights were established in advance for each year's evaluation. The final group composite evaluation results were then summarized for presentation to the Council to help them make informed project funding decisions.

Cost was not scored directly, but cost estimates were reviewed for completeness and realism. The Panel and Council used cost information to assess overall project cost-benefit. In some cases, partial or phased project funding was recommended.

Based on the project scores, projects in each category were assigned by the Panel to one of 3 tiers. Tier 1 consisted of top scoring projects that provided substantial benefits and that were recommended for funding. Tier 2 consisted of projects with lower overall benefit than tier 1, but with benefits above a minimum threshold established by the Panel. The lowest scoring projects that failed to achieve the minimum threshold benefit were assigned to tier 3 and were not recommended for funding. In addition, the Panel made project funding recommendations including a level of funding or the need for a feasibility project before full funding.

Adaptively Modify the Evaluation Framework

As the Council's restoration effort was expected to continue for 5 years, the evaluation system was designed to be readily modified by adjusting criteria weights or rating scales or by adding criteria or feasibility factors. To reflect the results of ongoing restoration efforts, natural recovery, or new information from ongoing studies, the Panel has adjusted the criteria weights annually, added new factors needed to

improve the decision analysis method, and modified the summary and recommendations format it provides to the Council.

Develop Lessons Learned

An ongoing effort is underway to capture lessons learned and determine how this evaluation process might be improved for future grant cycles or other applications. Members of the Team involved in the development of the decision support system, the Panel applying it, and Council receiving Panel recommendations were interviewed to help identify how the system performed and how it might be improved. Several modifications were made, including 1) addition of a minigrant program for projects less than $3,000, 2) addition of a new feasibility factor to capture issues related to "implementability," 3) conversion of rating scales for those criteria to be minimized so that scales are all increasing, 4) elimination of the research category, and 5) addition of a Council strategic planning effort.

DISCUSSION

The decision support system developed to comparatively evaluate Cantara restoration proposals has been applied in 3 grant cycles and has been effective in proposal reviews as reflected by the Council's recommendations (Table 1). For 1996 and 1997, the council declined to fund only 1 tier 1 project and only 2 tier 3 projects. Overall, 94% of the projects assigned to tier 1 received full or partial funding, whereas only 3% of the projects assigned to tier 3 received any funding. Most of the other inconsistencies occurred in tier 2 when the Council decided to provide no funding after the Panel recommended partial or full funding or decided to award partial funding when the Panel had recommended full funding. On occasion, the Council requested changes in the scope of projects or directed a phased project implementation.

The interactive system development process involved a series of discussion, voting, and review and adjustment sessions. The insight developed while quantifying judgements was extremely valuable in focusing group discussion on any remaining areas of controversy, identifying data gaps, clarifying tradeoffs, and suggesting methods for tailoring submitted projects to meet requirements.

One of the primary benefits provided by the decision analysis method was the structured framework for efficient group review. The structured approach helped link Trustee strategic values to the decision framework and grant program announcement. It established a clearly documented and defensible approach that informed stakeholders and grant program respondents of the evaluation factors and their relative importance. We believe this has contributed to the generation of better restoration proposals and decisions.

Although useful in informing the Council, the method was used as a decision analysis rather than a decision making tool. Several key decision elements were not considered in the present system. For example, it did not address allocating resources across categories or resources, as it was not clear how many proposals would be received in each category in any given year. In addition to the project funding

Table 1. Relationship between Technical Review Panel evaluations and Council funding decisions for 115 Cantara spill restoration proposals received during 1996 and 1997. Consistency reflects how consistent the Council's decisions were with respect to the Panel's specific recommendations related to specific subtasks, funding levels, or phased implementation.

Category	Panel benefit tier	Council funding Full	Part	None	Percent funded	Percent consistency
Restoration	I	5	1	0	100	83
	II	0	4	3	57	71
	III	1	0	15	6	94
Acquisition	I	2	2	0	100	100
	II	0	1	2	33	100
	III	0	0	5	0	100
Research	I	1	2	1	75	75
	II	0	3	11	21	71
	III	0	0	28	0	100
Education	I	0	3	0	100	100
	II	0	2	4	33	100
	III	0	1	8	11	89
Tier Totals	I	8	8	1	94	88
	II	0	10	20	33	83
	III	1	1	56	3	98
Total		9	19	77	24	91

decisions, the Council decided, annually, how to develop a balanced restoration program.

Based on the experience in applying the system, some "lessons learned" that may be useful for future restoration planning efforts were identified. These lessons include the importance of:

1) establishing a qualified and dedicated interdisciplinary team early in the NRDA process to allow adequate time to consider all important aspects of the restoration planning and to expedite restoration implementation;

2) selecting an appropriate method that addresses analytic requirements, is readily understood, and is documented for internal and external review;

3) focusing first on values, including those in charter documents and trustee agency policy, rather than alternatives to provide a better portfolio of alternatives to evaluate;

4) carefully developing evaluation criteria, criteria weights, and other factors in a manner consistent with the assumptions of the additive weighting model;

5) verifying and validating the model and any software used to implement it; and

6) adaptively managing the process as new information becomes available or requirements change.

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HINDSIGHT ANALYSIS FOR THE CANTARA SPILL NATURAL RESOURCE DAMAGE ASSESSMENT

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The 3 suggested phases of Natural Resource Damage Assessment (NRDA) under federal regulations are injury determination, injury quantification, and damage determination. As conceptualized in regulation, this process is straightforward. Implementation is complicated by numerous factors, including the need to quickly mobilize and collect ephemeral or time-critical data, safety concerns, the large scale of a comprehensive ecosystem assessment, administrative procedures, political and resource management considerations, impractical regulations, financial risk due to uncertainty that costs will be recovered, scientific studies that do not meet performance or time line expectations, and aggressive litigation tactics by the potentially responsible parties. In contrast to other large scale scientific endeavors, NRDA is designed within a litigation framework that ultimately will enable recovery of damages. Litigation objectives can greatly increase the cost and complexity of NRDA. Procedures to manage documents, confidentiality issues, litigation strategy, and court mandated deadlines significantly influence each decision made in the course of the damage assessment. The Cantara Spill NRDA successfully contributed to an out-of-court US$38 million settlement to fund recovery monitoring, restoration projects, and reimbursement of all costs of the NRDA. This experience can be instructive for future spill response efforts. To an extent that exceeds the usual requirements of applied resource management, scientists engaged in injury determination and quantification in anticipation of litigation must consistently apply rigorous standard scientific procedures such as identifying testable null hypotheses, power analysis, random sampling, quality assurance and quality control, and peer review. To this lexicon, we must add chain-of-custody, confidentiality and privilege, document management, discovery, stipulations, and litigation strategy.

INTRODUCTION

In response to the Cantara Spill of metam sodium (Garrison 2000), a Natural Resource Damage Assessment (NRDA) was initiated by trustee agencies pursuant to the Comprehensive Environmental Response Compensation and Liability Act (CERCLA). Trustees for the NRDA included the State of California Resources Agency acting through the Department of Fish and Game (CDFG), U.S. Department of Interior acting through the U.S. Fish and Wildlife Service (USFWS) and the U.S. Bureau of Reclamation which manages Shasta Lake. The lead trustee for the NRDA was the CDFG.

The NRDA included 3 principal stages: injury determination, injury quantification, and damage determination. Procedures for implementation, at the time of the incident, were described in regulation (Federal Register[1] 1991). In simple terms, injury determination means the confirmation that specific natural resources were injured by exposure to a hazardous substance. Injury quantification characterizes the extent of such injury, and damage determination estimates the economic value of the injuries. The injury determination and quantification phases serve the damage determination process by providing defensible information about the type and extent of ecosystem damage. In concept, an NRDA is a straightforward exercise to collect environmental and economic data for use in characterizing the biological and lost human use consequences of a hazardous material spill and then estimating the economic cost to restore and compensate for lost resources. In application, an NRDA can become tremendously complicated.

NATURAL RESOURCE DAMAGE ASSESSMENT

Injury Determination and Quantification

Response

The initial response to a spill of hazardous materials and adequacy of early data collection largely determine whether a successful NRDA can be implemented. This is particularly true where the toxic agent persists for only a short time or if baseline information is scanty. The collection of data immediately following the Cantara Spill was complicated by several factors.

Safety considerations - Potential exposure to contaminated air, water, and soil severely restricted access for 3 days and, to lesser extent, for several additional days. Biologists for the CDFG had neither the training nor protective clothing to access the river under those conditions. Deep water, steep terrain, poor access, hot weather, poor radio communication conditions, and poison oak were also significant safety issues. Problems with hazardous field conditions may be minimized by training, equipment, and improved communication technology (e.g., cellular telephones, radios).

Ephemeral/time-critical data - The window of opportunity for collection of data to document the initial injury was limited. Injured organisms were subject to several possible fates, which collectively reduced the likelihood that they would be observed. These mechanisms included decomposition, consumption by scavengers, accumulation in deep pools, fluvial transport out of the impact area, and deposition under rocks and debris. Injured terrestrial wildlife were particularly difficult to locate. They may have traveled some distance from the contaminated area before dying or were hidden in dense vegetation, nests, tree cavities, or caves (including crevices in bridges).

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[1] Federal Register. 1991. 56(82):19752-19773. 29 April 1991. Current regulations can be found at 43 C.F.R. §11 *et seq.*

Early collection of data serves both injury determination and quantification purposes. Without such data, attorneys for the potentially responsible parties (PRPs) are unlikely to stipulate to injury and can aggressively challenge any damage claim based upon inference. An effective alternative, where feasible, is to use in situ terrestrial bioassays placed in the path of an advancing spill. Recommended test organisms include those that can be rapidly collected (e.g., lizards) or purchased from domestic sources (e.g., rats, mice, and birds). The use of caged organisms where they will be exposed to toxic pathways and in uncontaminated control areas can be highly effective in reducing subsequent NRDA costs and litigation conflict.

The importance of an early and comprehensive response to identify and quantify injured aquatic and terrestrial organisms is also reduced if baseline data characterizing species richness, distribution, and abundance is available. Pre-spill information related to habitat quality is valuable for documentation of adverse changes attributable to the release of hazardous substances.

In addition, contamination of soil, water, and air may also be ephemeral. Documentation of such contamination is critical to demonstrating the pathways through which biological resources are injured.

Unpredictability - It was impossible to plan for all possibilities. Staff availability and unfamiliarity with CERCLA procedures reduced the effectiveness of the initial response. Incomplete information about the hazardous material in terms of its toxicity and fate in the subject environment also impaired the response.

Adequacy of control sites - One of the most difficult elements of an NRDA is the selection of comparable control sites for injury determination and quantification tasks. The Cantara Spill damaged the entire 66 km reach of the river between Cantara Loop and Lake Shasta (On 14 July 1991, the downstream 10.6 km of this reach was below the full pool elevation of Lake Shasta, which was drawn down due to drought). In addition, the 3.7 km reach above Cantara Loop to Box Canyon Dam was exposed to metam sodium and its breakdown products through the air. The preferred control of samples collected from the impact area immediately prior to the toxic discharge was not available. In the absence of pre-event sampling, real time photography during the spill, in situ bioassays, and samples of water and air collected before the toxic plume reaches specified locations in the area affected by the spill are especially useful. Data collected in advance of the spill (e.g., invertebrate and vegetation sampling) can be tremendously valuable when later compared to similar sampling efforts following exposure. Techniques for invertebrate sampling are readily available (CDFG[2] 1996).

Exposure Pathways

The NRDA regulations provide guidance for trustees to identify the pathways by which natural resources are exposed to hazardous materials. For the Cantara Spill, water, air and soil provided the pathways by which metam sodium and its breakdown

[2] CDFG. 1996. California stream bioassessment procedure. California Department of Fish and Game, Water Pollution Control Laboratory, Rancho Cordova, California, USA.

products reached biological resources. Breakdown products injured natural resources through the surface water pathway and volatilized from the river into the air where they underwent photolytic breakdown in sunlight. Toxic gases were concentrated over the plume but were also distributed by wind. Wind carried toxic gases down the Sacramento River canyon, laterally away from the river, and even up the canyon. Exposure of plants and animals to toxic gases was highly localized and pockets with relatively high concentrations remained for some time.

Shallow groundwater was polluted by toxins from the river and riparian soils were contaminated by deposition of toxic residues from the air and by rain which fell on 16 and 17 July 1991. Toxic residues from the air were deposited on and respired by vegetation and wildlife. Although the chemical degraded rapidly in the river and air, 1 breakdown product was detected for about 30 days in river sediments (CRWQCB[3] 1991). Taylor et al. (1994) found measurable changes in microbial communities in riparian soils more than a year after the Cantara Spill.

Natural Resource Damage Assessment Plan

The Trustees prepared draft (CDFG[4] 1991) and final (CDFG[5] 1993) NRDA plans that described the investigations to determine which resources were injured and estimate the type and extent of such injuries.

Damage Determination

The 3rd principal component of an NRDA is the damage determination phase. "The purpose of the damage determination phase is to establish the amount of money to be sought in compensation for injury to natural resources resulting from a discharge of oil or release of a hazardous substance" (Federal Register[1] 1991). Several techniques can be used to estimate the value of lost resources (e.g., Hanneson 1993; AFS 1992, 1993). This phase is of interest to the PRPs as results can yield economic liability.

The Trustees selected a technique known as the "contingent valuation method (CVM)." This method can be used to estimate the total value of lost resources in terms of both "use" and "non-use" (also referred to as "passive") values. The term "use" applies to services provided to the public by the ecosystem for which some direct measure is available (consumptive or non-consumptive). Examples include recreation (e.g., fishing, and kayaking), public park revenues, and education field trips. "Passive" or "non-use" values arise from services provided by the ecosystem to the public for which no direct active use is known. These are also described as the existence, intrinsic, bequest, or heritage values.

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[3] CRWQCB. 1991. Final water sampling report: Southern Pacific-Cantara Spill. California Regional Water Quality Control Board, Central Valley Region, Redding, California, USA.

[4] CDFG. 1991. Draft Natural Resource Damage Assessment Plan, Sacramento River: Cantara Spill, Shasta and Siskiyou counties, California. California Department of Fish and Game, Redding, California, USA.

[5] CDFG. 1993. Final Natural Resource Damage Assessment Plan, Shasta and Siskiyou counties, California. California Department of Fish and Game, Redding, California USA.

In the CVM, a conservative description of the ecosystem damage is developed along with recovery and restoration scenarios and possible means to prevent similar events in the future. This is presented to a representative sample of the public and a statistically valid economic estimate is generated, using survey and statistical techniques, to support the trustee's damage claim.

Contingent valuation method surveys can be expensive to perform and typically are implemented only in large-scale damage cases. Successful use of the methodology depends upon scientifically sound injury determination and quantification studies in addition to valid survey procedures. The PRPs could potentially undermine credibility of a CVM if any of the underlying characterizations about ecosystem injury can be proven to be incorrect or unreliable. Thus, the discovery process became an important avenue for the PRPs to learn the strength of the underlying natural science work.

ADMINISTRATIVE PROCEDURES

Organization

Injury assessment was divided into 3 functional areas: 1) vegetation, 2) terrestrial wildlife, and 3) aquatics. Teams representing each functional area began fieldwork to characterize and quantify injury as early as safety considerations allowed. Based on field observations and consultations with other scientists, the Trustees identified numerous natural resources at risk from the Cantara Spill. In accordance with NRDA regulations, the Trustees designed, or asked university, agency, or consulting scientists to design injury determination and quantification studies.

Shortly after the Cantara Spill, it became apparent that the continued diversion of existing staff to the NRDA would have unacceptable long-term impacts on other CDFG programs. In July 1992, the Cantara Program was established at the Redding, California, office of the CDFG. Staff to implement the NRDA included a supervisor, wildlife and aquatic team leaders, a plant ecologist, administrative support staff, temporary employees, and a substantial budget for external contracts for NRDA tasks. Additional legal staff were hired in CDFG headquarters in Sacramento. In hindsight, the program could have been much more effective with a full-time biostatistician and a scientific coordinator for the injury determination and quantification tasks. Consultants were contracted to perform some of these services but the results were inferior to having dedicated full-time staff. The NRDA was well under way before serious efforts began to integrate results of all tasks into a synergistic process. The ultimate use of NRDA to tell a defensible story of ecosystem injury and recovery for damage determination and litigation requires the integration of methods and results from the component tasks.

Funding

The State of California budgeted $12.4 million for NRDA expenses from July 1991 through June 1994. This amount would have been insufficient to complete and litigate a comprehensive NRDA that would have withstood an aggressive challenge

in court. Another several million dollars would have been necessary, mainly for damage determination and litigation costs. The level of funding needed for an NRDA is a function of many factors, including the complexity of impacts to the environment and public as well the litigious nature of the PRPs.

Contracting

Complex bureaucratic difficulties involved in executing the NRDA (including contracting, hiring, and purchasing) were overcome by recognizing that it was one of CDFG's highest priorities and by outstanding administrative staff efforts.

Consultant performance ranged from poor to excellent. Where performance was unsatisfactory, 1 or more of the following problems were present: 1) expectations for deliverable products and time lines were poorly articulated; 2) litigation objectives such as confidentiality, discovery, and court mandated deadlines constrained scientific inquiry and communication; 3) the consultant was overcommitted to other projects and unable to meet expected time lines; 4) the consultant viewed the NRDA as a convenient fund source for their own research objectives and tended to stray from agreed upon work plans; 5) the consultant failed to use proper statistical and quality assurance procedures; 6) the necessary linkage between study design and regulatory requirements was not clearly understood by the consultant.

For science to serve litigation objectives, NRDA studies must ultimately be rigorous, exhaustive, and defensible. Elements of a successful NRDA strategy should include clear contract expectations, communication between investigators, peer review, and ultimate publication of results. There are considerable short-term disadvantages when attorneys for the defendants obtain information about the NRDA and the Trustees must be prepared to devote sufficient legal and public information resources to counter PRP tactics.

Attorneys, and others, perceive that research scientists associated with universities perform higher quality work than government employees and raise the credibility of the party to which they lend their efforts. However, there was no indication during the Cantara NRDA that university scientists performed better in terms of timeliness, scientific rigor, or promptness of publication than government employees or private consultants not associated with universities.

STRATEGY

Trustees

Adherence to Regulations

Of the various laws requiring PRPs to effect recovery of the injured resources, CERCLA was considered by the Trustees to be the most important.

Regulations for the implementation of NRDA provide procedures for trustees to assess and recover damages for injured natural resources. These procedures are not mandatory; however, CERCLA (Federal Register[1] 1991) provides a "rebuttable

presumption" of correctness for the assessment when trustees follow the regulations. The PRPs then have the burden of effectively rebutting results of the NRDA. However, there is no precedent for this as it has not yet been tested in a court trial. Even so, attorneys for trustees will likely wish to obtain the potentially useful stratagem of the "rebuttable presumption" for litigation.

The regulations provide useful guidance for determining and quantifying injury to aquatic resources. Guidance for documenting impacts to terrestrial wildlife and vegetation is relatively limited and oriented mainly to injury determination and quantification for sites where contamination is persistent and ecosystem recovery is slow.

The regulations include several unrealistic recommendations. For example, they state that injury determination must be complete before initiating injury quantification (Federal Register[1] 1991). However, ephemeral data may need to be collected for quantification studies before the injury determination phase is complete. Court mandated deadlines may make it impractical to conduct all work sequentially and some determination tasks may be deferred with the expectation that negotiated stipulations may make the studies unnecessary. Another unrealistic element of the regulations is direction to invite the PRPs to participate in development of the NRDA (Federal Register[1] 1991). In most cases the interests of the PRPs will be inconsistent with a comprehensive NRDA that would account for the full economic value of all injuries.

Rigorous and systematic implementation of an NRDA is essential to success. Despite the problems identified previously, the experience gained following the Cantara Spill suggests that scientists should carefully follow the model for rigorous injury determination and quantification outlined in the regulations. However, minor deviations and additions to the procedures outlined in regulations should be expected.

Damage Occurred to an Entire Ecosystem

The Trustees believed that the NRDA should describe injury to the whole ecosystem, regardless of whether direct economic measures of the value of a particular resource were available. Metam sodium and its breakdown products were highly toxic to all organisms in the river, but toxicity to terrestrial wildlife was much less. This approach required an NRDA that characterized injuries to all trophic levels, from periphyton to raptors.

Damage Determination

The CVM of estimating damages depends upon presenting a conservative but comprehensive description of the injuries to survey respondents. This requires an equally comprehensive set of studies to validate the presentation. In the NRDA process, science (natural and social) provides the basis for estimating economic damages and supporting subsequent litigation. The injury determination, injury quantification, and damage determination phases are complementary and essential to one another.

Potentially Responsible Parties

Strategies

Strategies used by the PRPs were designed and implemented by attorneys. Some of the PRPs named in the state and federal civil litigation were named more than once in their various incarnations as corporations, holding companies, and subsidiaries. This is important because the PRPs might otherwise play a shell game with responsibility for their actions. In this case, Southern Pacific Transportation Company (SP) was the most active PRP.

Although the CERCLA NRDA regulations specify procedures for collaboration of trustees and PRPs in development of an NRDA (Federal Register[1] 1991), cooperation can be problematic because it is not typically in the PRPs' economic interest to ensure that a comprehensive assessment which characterizes the full extent of ecosystem and lost use damage is completed. Instead, their interest in limiting economic liability may best be served by preventing the NRDA from being completed or by reducing the integrity of the NRDA.

Alternative Science

Southern Pacific retained consulting scientists to perform services including:

Monitor NRDA task fieldwork - Scientists employed by SP accompanied or followed trustee scientists as they collected field data. They collected their own samples, videotaped data collection efforts by the Trustees, and took notes on techniques used for data collection.

Independent studies - In addition to collecting comparative data as they monitored trustee scientists, SP representatives performed studies to develop their own information relating injury determination and quantification.

Litigation support - During the discovery phase of litigation, the Trustees received a large number of interrogatories and requests for admissions. Most of these were intended to examine or challenge the adequacy of NRDA tasks and were crafted with the assistance of scientists.

Advocacy - Attorneys and scientists retained by SP made frequent presentations of their version of the Cantara Spill impacts to the State Fish and Game Commission, local governments, county Fish and Game Commissions, chambers of commerce, and the media. They said that 1) impacts of the Cantara Spill were overstated by the Trustees, 2) the CDFG impeded recovery of the river ecosystem by their refusal to stock hatchery raised trout, 3) NRDA tasks were poorly designed and implemented by incompetent scientists, and 4) the refusal to restore the ecosystem by stocking trout was part of a plan by the Trustees to increase the damage claim pursued in litigation and deprive the public of "family" fishing opportunities.

Impede NRDA Implementation

A common component of a litigation strategy is to overburden one's opponent so that the tasks that are most important to ultimate success cannot be accomplished.

There were several diversions by the PRPs that seemed designed to prevent complete implementation of the NRDA. One tactic was an offer by SP to fund and implement certain NRDA tasks. Negotiations over scope of work, implementation, oversight, and cost continued for months, although SP ultimately did not fund any of the NRDA tasks. The negotiations consumed time and staff resources, delayed task implementation, and provided information on the proposed objectives of the studies.

A 2nd tactic related to the development of potential stipulations. A stipulation is an agreement on the facts negotiated between the parties. The purpose is to identify issues that are not in dispute so that resources can be focused elsewhere. Injury determination is an area where stipulations can be especially useful. The Trustees proposed numerous stipulations intended to establish various facts that would not be disputed in following litigation. Negotiations over stipulations occurred sporadically over 2 years and required considerable staff effort in a process that ultimately did not produce any useful agreements.

A 3rd tactic by SP had significant potential to terminate the NRDA before it produced useful results by lobbying the legislature in June and July 1993 to terminate funding before the NRDA was complete, even though nearly $9 million had already been allocated. The CDFG ultimately convinced the legislature that funding for the NRDA was a good investment and an additional $3 million was approved.

LITIGATION

On 13 July 1992, the State of California filed a lawsuit against the PRPs, citing CERCLA, several sections of the Fish and Game Code, the California Water Code, and California Common Law as causes of action. On 17 December 1992, the Federal government filed a similar suit in United States District Court under CERCLA, the Clean Water Act, and the Rivers and Harbors Act.

Ecological investigations are typically intended to improve our understanding of processes or to inform management decisions. These investigations can also serve litigation by demonstrating that one side is better able to meet the legal burden of proof and thus may prevail in court and collect monetary damages. This litigation support role is unfamiliar and uncomfortable for many scientists because the objectives of scientific inquiry are quite different from the objectives of litigation. Success in litigation is achieved when an alternate view of events has been discredited. In contrast, a successful scientific inquiry is achieved when agreement is reached about the probability of cause and effect.

It is the responsibility of an attorney to present complex natural phenomena in simple discrete terms, enabling a judge or a jury to reach a decision favoring one side over another. Although scientists often feel that a conclusion cannot be reached without collection and analysis of additional information, that opportunity may not be available. Litigation does not afford the endless collection of information. Instead, court mandated deadlines impose arbitrary endpoints.

The process known as "discovery" precedes an actual trial and provides opponents in the litigation an opportunity to learn the facts available to the other side. This process can result in the premature disclosure of research methods and findings before they have been adequately peer reviewed. It can also be very disruptive to the

performance of NRDA studies, a fact that is not overlooked by attorneys. Every piece of information that becomes available to opposing counsel becomes raw material for new efforts to disrupt completion of the scientific work. This creates powerful motivation to maintain confidentiality. Discovery ultimately has considerable value where the trustee's legal case is supported by strong scientific studies. For the Cantara Spill, a favorable settlement in the amount of US$38 million was reached following extensive discovery during which the PRPs had the opportunity to review preliminary findings of the NRDA tasks.

Litigation between the Trustees and the PRPs was further complicated by the intervention of 6 sportfishing and environmental organizations. Early settlement proposals by the intervenors asserted that they should control the use of most settlement funds. The Trustees were unable to agree with this outcome and moved to have the intervenors dismissed as a party to the litigation. This process lasted until December 1994, when the court determined that the intervenors did not have legal standing and dismissed them from the litigation. This decision was appealed by the intervenors. In the interests of resolving the litigation and moving forward with restoration, the Trustees negotiated a settlement with the intervenors in August 1995. The intervenors received $265,000 for proposed railroad safety monitoring activities and an advisory role on the Cantara Trustee Council. With this settlement it was possible to move forward with establishment of the Cantara Trustee Council and restoration activities. Negotiations to resolve the intervenors interests delayed implementation of restoration actions for more than 1 year after agreement on settlement of damages was reached with the PRPs.

RESOURCE MANAGEMENT

Fisheries

Prior to 14 July 1991, the aquatic ecosystem of the Sacramento River supported a popular recreational fishery for wild and catchable rainbow trout, *Oncorhynchus mykiss*. A few brown trout, *Salmo trutta*, and brook trout, *Salvelinus fontinalis*, were also present, along with Alabama spotted bass, *Micropterus punctulatus*, and smallmouth bass, *M. dolomieui*, in the river near Lake Shasta. The loss of this fishery had a major economic impact and was compounded by the perception of some people that the area was contaminated and unsafe for months or even years after metam sodium and its breakdown products were undetectable.

Some segments of the public and the PRPs demanded that the CDFG immediately stock the river with hatchery trout to restore the fishery and provide fishing opportunities. Instead, the Fish and Game Commission closed the river and tributaries to fishing and the CDFG deferred a decision on stocking while considering the best approach to achieve recovery of the entire ecosystem. This cautious approach was criticized by local businesses dependent upon tourism and by the PRPs. The PRPs noted that following a lesser spill in 1976, the CDFG had "restored" fishing opportunity by stocking hatchery trout and accused the Trustees of stalling in order to increase the damage claim under the NRDA.

The Fish and Game Commission kept the upper Sacramento River closed to angling until April 1994. This approach included the deferral of stocking domestic trout, based on the CDFG's strategy that sought to 1) minimize competition and genetic introgression between wild and domestic trout, 2) avoid incidental harvest of wild trout, and 3) minimize the attraction and premature recovery of predators.

The CDFG believed that stocking domestic trout would not facilitate recovery of a self-sustaining trout population. Monitoring indicated that wild trout recovery was proceeding rapidly relative to recovery of other aquatic organisms.

Throughout this period, attorneys and biologists employed by SP appeared at most Fish and Game Commission meetings to lobby for restoration of hatchery trout stocking and angling and take every opportunity to publicly present their views. Responding to these presentations became a significant workload for CDFG staff, which detracted from the resources available to conduct the NRDA.

Another segment of the angling public wanted a slower restoration of fishing opportunity, preferably limited to catch-and-release angling with barbless artificial lures. The debate between these interests and those arguing for restoration of "family" fishing, preferably with bait and barbed hooks, became quite polarized.

Fishery management planning during the recovery period began in January 1992, when interested parties advanced a proposal (California Trout et al.[6] 1992) for management of the upper Sacramento River. In March 1992, the CDFG released a proposed management plan (CDFG[7] 1992) that included (1) highly protective conditions for streambed alteration agreements on the upper Sacramento River and its tributaries; (2) a recommendation to the Fish and Game Commission that the upper Sacramento River and its tributaries remain closed to fishing through 1992; (3) continued closure of the upper Sacramento River and its tributaries to suction dredging; (4) review of projects subject to the California Environmental Quality Act and Forest Practices Act in the watershed for purposes of making protective recommendations to lead agencies; (5) continued review and involvement in the operations of hydroelectric projects in the watershed; (6) increased stocking of hatchery trout into other nearby waters; and 7) an experimental fisheries management program to: artificially propagate wild trout from brood stock collected upstream from the Cantara Spill site and stock the progeny in the river, relocate adult wild trout from above the Cantara Spill site to 1 or more downstream locations; and monitor recovery of nongame species to determine if a barrier was warranted to prevent emigration from Shasta Lake.

The first 6 measures were successfully implemented, in part through the activities of a Fish and Game Warden hired in July 1992 whose sole responsibility was to offer fish and wildlife protection to the upper Sacramento River and its tributaries during the recovery process.

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[6] California Trout, Shasta-Cascade Wonderland Association, Southern Pacific Transportation Company, and Trout Unlimited. 1992. Proposed elements of an Upper Sacramento River Recovery Plan.

[7] CDFG. 1992. 1992 Fishery Management Plan: Upper Sacramento River, Shasta/Siskiyou counties, California. California Department of Fish and Game. Sacramento, California, USA.

The experimental fisheries management program began in March 1992. The 1st element, collection and propagation of wild brood stock, was implemented and resulted in stocking about 7,000 fingerling rainbow trout in August 1993. The 2nd element was not implemented because of low numbers of wild trout at the collection sites. The nongame fish assessment led to the conclusion that the proposed barrier to restrict emigration was unnecessary.

On 26 March 1993, the CDFG advanced another management plan (CDFG[8] 1993) that reported on implementation of previous measures and presented recommendations for the following year. Management recommendations for 1993 included the first 6 components of the 1992 plan, continuation of the nongame fish assessment, and evaluation of the utility and potential for the addition of spawning gravel to enhance future spawning and recruitment of wild trout.

The fishing closure on the upper Sacramento River ended on 30 April 1994. Restrictive regulations were enacted for 1994–1997 that provided for catch-and-release fishing with artificial lures and barbless hooks for all but 10 km of the river that runs through Dunsmuir. In this section anglers were allowed to use bait or artificial lures, both with barbless hooks. Approximately 4,170 kg of hatchery trout were stocked each year into the Dunsmuir reach.

In the interval between the Cantara Spill and settlement of litigation with the PRPs, several studies intended primarily to quantify injury for the NRDA served the secondary purpose of informing resource management decisions by providing estimates of the prespill populations and recovery status (Turek[9] 1999).

Fishing regulations continue to evolve for the upper Sacramento River. Regulations may never be identical to those that immediately preceded the Cantara Spill. Hatchery production has declined for budget reasons, the culture of the fishing public has changed to include a larger proportion of catch-and-release anglers, and businesses in the affected area have become more aware of the economic benefits that derive from attracting catch-and release anglers to the area.

Suction Dredging

Under §5653 of the Fish and Game Code, the CDFG may regulate the location of suction dredge operations and restrict the type of equipment used. Because suction dredging potentially adversely affects benthic invertebrates, fish, and downstream spawning gravels, suction dredging was prohibited on the upper Sacramento River and its tributaries after the Cantara Spill.

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[8] CDFG. 1993. 1993 Fisheries Management Plan for the Upper Sacramento River (Box Canyon Dam to Shasta Lake), Shasta/Siskiyou counties, California. California Department of Fish and Game, Sacramento, California, USA.

[9] Turek, S.M. 1999. The use of angler surveys in managing the recovery of the upper Sacramento River wild trout fishery. Unpublished technical report submitted to the Cantara Trustee Council, February 1998. California Department of Fish and Game, Redding, California, USA.

The closure for suction dredging was modified in 1995 to allow for operations under "special" permits that enable the CDFG to condition the permit with restrictions on how and when the activity may occur to minimize potential adverse impacts.

CONCLUSION

Successful implementation of an NRDA requires that the scientists who perform the injury determination and quantification tasks understand the complex framework in which their work is done. Although injury determination and quantification studies may contribute new insights into species biology and ecosystem processes, the reason for these studies is to provide information for other activities, such as damage determination, litigation, and ultimately restoration.

To an extent that exceeds the usual requirements of applied resource management, scientists engaged in NRDA must consistently apply rigorous scientific procedures including the identification of testable null hypotheses, power analysis, random sampling, quality assurance/quality control, and peer review. To this lexicon, scientists must add new terms such as chain of custody, confidentiality and privilege, document management, discovery, stipulations, and litigation strategy.

A successful NRDA is based upon sound science, but this will not guarantee recovery of damages or the ecosystem. There are many ways for trustees to fail in an NRDA. In addition to conducting a rigorous damage assessment, the trustees must also ensure adequate budgets, excel at implementing administrative procedures, participate in political processes, and provide legal counsel with the information needed to prevail over the PRPs in court. These burdens make the process extremely difficult and trustees must carefully evaluate their options before entering into an NRDA. The Cantara Spill NRDA is an example of the successful integration of science with administrative, political, and legal processes to recover damages for injured natural resources. The net cost of the NRDA for the public was zero, a significant amount of new information was collected on the injured resources, monitoring and restoration activities are in progress, and the ecosystem is nearing recovery. In addition, projects are being implemented that will compensate the public for the interim lost uses of the river due to the Cantara Spill.

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